Low heterospecific pollen deposition on native plant stigmas despite high exotic abundance in southern Ontario meadows

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

In southern Ontario many meadow remnants are found within protected areas, including Conservation Authorities. These meadows occupy a fraction of their former area and are typically dominated by exotic species, yet they are an important ecological component within the landscape matrix. Meadows provide habitat and forage resources for many pollinator species but the relationship between pollen deposition and the presence of exotic/native species is currently unknown. This study aimed to determine the composition and quantity of pollen deposition on stigmas of exotic and native plants species in southern Ontario meadows to establish a baseline for future meadow pollen deposition studies. Furthermore, this study suggests monitoring programs and restoration techniques to help continually assess plant-pollinator interactions within Ontario's Conservation Authorities. Pollen deposition and pollinator visits were assessed from June-September 2011 in four meadows located in the Credit Valley Conservation Authority (2) and Toronto and Region Conservation Authority (2). Transects and quadrats were utilized for stigma collection, pollinator observations, and percent plant cover, while the Shannon Weiner Diversity Index and Pielou's Evenness Index were used to assess the diversity of deposited pollen. Exotic species represented 68 % of plants sampled. While native plant stigmas were chiefly deposited with conspecific pollen they only represented 32 % of the species within meadows. The highest average pollen diversity (1.03 ± 0.3SE) was observed on exotic *Cirsium* species stigmas, while *Hypericum perforatum* and *Cirsium* species, both exotic, demonstrated significantly different diversity indices ($P < 0.05$) due to heterospecific pollen transfer. *Potentilla recta* and *Melilotus alba* were only observed at one site, yet had high pollen diversity (1.16 and 0.97, respectively). The heterospecific pollen found on *Potentilla recta, Melilotus alba*, and *Cirsium* species stigmas originated from other exotic species rather than native species. These

results indicate that although there is a high abundance of exotic species in these meadows, the pollination, and thus reproductive success, of native species is likely not impeded by heterospecific pollen transfer.

Given the high number of exotic species found within meadows, it is unlikely that these meadows can be restored to an entirely native ecosystem and the lack of heterospecific pollen deposition found on native plant stigmas suggests that native plant reproduction is not currently impeded. Attempts to reduce exotic species' populations in an intense and rapid manner would not only be expensive and likely futile but may also cause pollinator population crashes unless mass plantings (restoration) of native plants and their entire soil community were successful. One alternative is to take a laddered approach of targeting those exotic plants which are hyper-abundant and/or which disrupt ecosystem services and gradually reducing their numbers by supplanting them with native species with similar floral characteristics to avoid a pollinator population crash. In southern Ontario's landscape, there should be a conscious effort to maintain a matrix (a sere of meadows, shrublands, and multi-aged forests) to support a diversity of flora and fauna.

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1.0 Introduction

Globally, biodiversity is being lost at an alarming rate - 1000 times faster than the background normal extinction rate – thereby potentially threatening human health and well-being (International Union for Conservation of Nature (IUCN 2010b). Biodiversity is defined as the diversity of all organisms and species between ecosystems and within them (IUCN 2000). The IUCN lists habitat loss as the greatest threat to global biodiversity (IUCN 2010b). Generally, habitats with decreased biodiversity have a decreased capacity to withstand anthropogenic pressures, such as urbanization and fragmentation (Vitousek et al. 1997, Henwood 1998). By disrupting these natural systems, humans lose the ecosystem services they provide, which can result in a decreased quality of life. As global populations rise there is concern over maintaining ecosystems and their biodiversity.

Meadows are among several ecosystems that are declining in area (Henwood 1998, Waesch and Becker 2009, Halpern et al. 2012). They are found on all continents except Antarctica and are one of the least protected ecosystems globally (Henwood 1998). Meadows, grasslands, and prairies are all used to describe areas dominated by forbs and graminoids; meadows are generally successional seres that eventually lead to the establishment of forests (Henwood 1998). Similar to grasslands and prairies, meadows are defined in the southern Ontario Ecological Land Classification (ELC) as "tree and shrub cover < 25 %; open herbaceous communities; where cover varies from scattered and patchy to continuous…dominated by grasses and/or broadleaf species, forbs (Ministry of Natural Resources 2008)." Distinguishing meadows and prairies from one another is difficult due to similar species compositions (Delany et al. 2000) and soil conditions (Ministry of Natural Resources 1998), therefore meadows shall be defined as an open habitat dominated by forbs and grasses.

Urbanization and agriculture are the two main threats to meadow habitat loss (Henwood 1998, Cane and Tepedino 2001, Winfree et al. 2009); however, anthropogenically planted trees which increase the rate of succession (Van Auken 2000) as well as fire suppression (Norman and Taylor 2005) both contribute to further global meadow declines. Meadows are easily converted to agricultural fields or urbanized development projects such as buildings, parking lots, or housing developments due to their relatively flat surfaces, lack of trees, and fertile soils (Henwood 1998). In addition to the direct loss of meadow habitat, plant species extirpation may also occur at higher rates in urbanized landscapes due to decreased population survival in smaller fragmented meadows (Fischer and Stocklin 1997). Furthermore, the genetic diversity of plant species may be reduced in remaining fragmented meadows (Young et al. 1996, Honnay and Jacquemyn 2007), thereby potentially reducing plant fitness (Keller and Waller 2002) and leading to further declines in meadow plant populations.

Loss of meadow habitats can potentially have negative effects on pollinators through loss of food resources and nesting sites (Forup and Memmott 2005, Kremen et al. 2007, Westphal et al. 2008, Aldridge et al. 2011).[1] These potential effects on pollinators threaten our food systems (Kremen et al. 2007), as well as, the ecosystem services humans rely on (Kearns et al. 1998, Packer 2010) through decreased animal-assisted pollination. Approximately 60-80 % of all plants require animal-assisted pollination to sustain their populations (Kearns et al. 1998, Ashman et al. 2004) and the loss of meadow habitat is cited as a large contributor to general pollinator declines (IUCN 2010a). Plants and pollinators rely on one another for reproduction and typically create facilitative or mutualistic interactions with one another, where the survival of

[1] For readers needing a formal definition, pollinators are animal vectors that transport pollen from one individual to another individual of the same flowering species to facilitate plant species reproduction.

one is related to the survival of another (Waser et al. 1996, Richardson et al. 2000a, Moragues and Traveset 2005, Bartomeus et al. 2008).

Currently, there is concern over worldwide pollinator declines (Packer 2010, Potts et al. 2010). Land use intensification may reduce pollinator abundances and diversity. Urbanization can reduce the availability of nesting sites due to increased impervious surfaces (Jha and Kremen 2013) and reduce the number and size of foraging areas available for pollinators (Winfree et al. 2009), thereby potentially negatively affecting pollinator populations. Some pollinator species' foraging patterns may be limited and/or display site fidelity within fragmented landscapes (Bhattacharya et al. 2003, Dorchin et al. 2013) thereby potentially reducing access to resources.

Plant-pollinator community studies largely focus on *Apis mellifera* (European honey bee) and *Bombus* (bumblebee) species due to their importance in agriculture (Gordon et al. 1998, Menz et al. 2011). Few studies have examined the responses of wild native bee populations to urbanization and fragmentation (Cane 2001) even though wild native bee populations, along with other native pollinator populations, are largely responsible for maintaining native plant populations (O'Toole 1993, Biesmeijer et al. 2006) and consequently maintaining ecosystem biodiversity. While declines in pollinator populations have been long documented in Europe (Biesmeijer et al. 2006, Rasmont et al. 2006, Food and Agriculture Organization 2008, Packer 2010), recently Cameron et al. (2011) discovered declines in North American pollinator populations over the past 20-30 years. With concern over worldwide pollinator declines (Potts et al. 2010) and specific concerns over North American pollinator declines (Cameron et al. 2011) there is a need to study current plant-pollinator interactions within urbanized landscapes to monitor, conserve, and potentially restore plant-pollinator communities in order to reduce further biodiversity loss.

Studies that have focused on plant-pollinator communities cite year-to-year variation as a hindrance for characterizing plant-pollinator systems and suggest long term studies to adequately assess community changes (Bjerknes et al. 2007). Long term monitoring programs are time consuming and are frequently reduced or discontinued (Caughlan and Oakley 2001, Government of Canada 2012), yet they provide conservation and ecological restoration practitioners with necessary and valuable information that enables them to execute successful conservation and restoration programs (Vaughan et al. 2001, Fancy et al. 2009). Continued declining biodiversity can result in increased extirpation and extinction rates, including a projected pollination decrease, therefore long term monitoring programs are needed to further understand spatial and temporal variations of plant-pollinator communities within pressured ecosystems.

1.1 Meadows within Ontario

Although Canada's overall population density is low, there has been an increasing trend in the growth of urban centers. Specifically, as of 1996, 83.3 % of Ontarians lived within urban centres, consisting of 100,000 people or more (Statistics Canada 2000). The majority of urbanization in Ontario has occurred within the southern region (Environmental Commissioner of Ontario 2012), which poses a threat to the biodiversity in southern Ontario. Historically, Ontario's landscape was mostly covered by forest but scattered with meadows and prairies (Figure 1 in Delany et al. 2000). Meadows existed prior to anthropogenic influences through drought and flooding, as well as forest fires (Packard 1997, Delany et al. 2000). It has been suggested that repeated burning by aboriginal peoples (Wood 1961, Lumsden 1966), enhanced meadow and prairie ecosystem creation. Since the settlement of Europeans in southern Ontario the majority of meadows have been removed from the landscape for settlement or agriculture, resulting in few continuous meadows. Currently, meadows comprise approximately 10 % of

conservation land in southern Ontario Conservation Authorities (Toronto and Region Conservation Authority 2007, Credit Valley Conservation 2009).

Pollinators can influence the reproductive success (seed production) of plants through pollen deposition origin and quantity. Several plant species are often pollen limited (Burd 1994, Ashman et al. 2004), where there are more ovules available for reproduction than pollen grains deposited, as the number of conspecific pollen (pollen originating from the same species) grains deposited on a stigma influences the resulting seed set (Morales and Traveset 2008). The seed set can be further impacted with the depositional presence of heterospecific pollen (pollen originating from a different species) resulting in reduce seed sets through stigma clogging (Galen and Gregory 1989, Proctor et al. 1996), chemical interference (allelopathy) (Sukhada and Jayachandra 1980, Murphy and Aarssen 1995b), and stylar clogging or inhibition (Brown and Mitchell 2001). Heterospecific pollen transfer can occur between sympatric species in undisturbed habitats (Levin and Kerster 1967) without the presence of invasive or exotic species.

With declining trends in North American pollinators, as well as increasing urbanization, meadow ecosystems within southern Ontario and the specific plant-pollinator interactions within them are threatened. Understanding plant-pollinator systems regionally is necessary to determine long term trends, as well as, protect the biodiversity within the limited green spaces found in urbanized landscapes.

1.2 Objectives of thesis

Overall there is limited information on pollen deposition on native forbs in Ontario meadows, thus the primary research question is do pollen grains from exotic or invasive species represent the majority of pollen deposited on native forbs in southern Ontario meadows? The overall objective of this study is to determine the composition of pollen deposited on stigmas in

southern Ontario meadows thereby highlighting potential long term threats to native meadow species by invasive or exotic species in southern Ontario. The specific objectives are to: 1) establish a general baseline knowledge of pollen deposition on stigmas of forbs within meadows in southern Ontario, which will aid in the creation of a plant-pollinator interactions monitoring program; 2) Identify conspecific and heterospecific pollen deposition, with emphasis on native and exotic species; 3) Utilize the deposition information to identify potential threats to southern Ontario meadows that exist in current plant communities; 4) Make recommendations for the restoration and/or management of southern Ontario meadows based on plant-pollinator interactions.

2.0 Literature review

The following section (2.0) consists of a review of literature on pollination biology and ecology; specifically, characteristics that influence conspecific and heterospecific pollen deposition to determine how invasive or exotic species may affect the pollination ecology of meadow ecosystems. Section 2.5 briefly introduces the state of meadow conservation in southern Ontario with a focus on Conservation Authorities.

2.1 Pollination ecology

Pollinators can be divided into two broad categories, specialists and generalists. Generalist pollinators have physical characteristics that allow them to access nectar and pollen from a variety of plants species (Seeley 1985) and are often attracted to large food rewards, as well as high density, high flowering plants (Lopezaraiza–Mikel et al. 2007). Specialists have physical characteristics that allow them to access nectar or gather pollen from specific flower morphologies (Seeley 1985, Armbruster et al. 1994). Nectar is comprised of sugar and water and is used for nutrition during offspring development (Roulston et al. 2000a). Pollen is also a source of nutrients during offspring development and is comprised of proteins (in varying quantities) and lipids (Roulston et al. 2000a, Roulston and Cane 2002). Specialist pollinators usually visit only one or a few specific plants with compatible physical characteristics to access nectar and pollen (Roulston et al. 2000a). Although specialists and generalist pollinators can occur within a plant-pollinator community, most plant species rely on generalist pollinator interactions (Waser et al. 1996).

Plants use different strategies to attract pollinators, such as high nectar rewards (Chittka and Schurkens 2001), scent (Andersson 2003), and attractive floral displays (Briscoe and Chittka

2001, Molleman et al. 2005). Regardless of the attraction mechanism, once pollinators visit a flower pollen often adheres to their bodies (Michener 2000). As pollinators travel to different individual plants of the same species, they transport conspecific pollen grains with them, which are deposited onto flowers' stigmas (Michener 2000). Conspecific pollen is pollen originating from the same plant species, whereas heterospecific pollen is pollen originating from a different plant species. Once conspecific pollen is deposited, pollen may create pollen tubes and eventually reach ovules to create seeds (Proctor et al. 1996). Sometimes pollen from the same individual will be deposited on its' own stigma, termed geitonogamy (de Jong et al. 1993) . If geitonogamy occurs and the plant is self-incompatible, it will be unable to reproduce with its own pollen (de Jong et al. 1993)**.** Many plants require pollen from different individuals of the same species to reproduce (self-incompatible). Conversely, some plants are self-compatible, where they can use pollen from themselves to reproduce**.** Plants that are self-compatible do not require pollinators for reproduction; however, higher seed sets have been found in self-compatible plants that are supplemented by outcrossed conspecific pollen (Proctor et al. 1996) and thereby making pollinators important for self-compatible plants**.**

Pollinators are pivotal to the reproduction of most plants to deliver genetic material to individuals. All of the different floral strategies are created to entice pollinators to only visit their own species' flowers, delivering their conspecific pollen, and ultimately reproducing. Some pollinators may exhibit pollinator constancy to specific plant species (Michener 2000); pollinator constancy is the tendency for a pollinator to visit one plant species for nectar or pollen within a foraging session even within the presence of other rewarding plant species (Waser 1986, Goulson 1994, Chittka et al. 1999). Pollinator constancy can aid in conspecific pollen transfer when there are several co-flowering species in an area because pollen attached to their bodies is

from only one plant species, thereby increasing the likelihood for successful plant reproduction. Flower colour is usually an important floral trait that promotes constancy (Hill et al. 1997, Keasar et al. 1997) but other traits such as size, scent, and floral shape can illicit foraging constancy (Keasar et al. 1997, Andersson 2003, Gegear and Laverty 2005). Additionally, constancy can be increased with increasing floral trait differences within a group of plant species (Gegear and Laverty 2005). There are several hypothesized variables that aid in explaining constancy choice; however, no one theory can explain when pollinators do or do not display constancy foraging behaviour (Chittka et al. 1999). Pollinator constancy can manifest on pollen species found on a stigma and/or pollen species found on a pollinator's body, each highlighting which plant species pollinators have visited (Goulson 1994, Pernal and Currie 2001, Fang and Shuang-Quan 2013). Pollen deposition on stigmas can highlight which floral resources are visited within foraging sessions by pollinators. Pollen deposition may highlight if any one floral species is highly visited and therefore represents the majority of pollen deposited on stigmas or if pollinators are generally depositing conspecific pollen grains on flowers they visit.

2.2 Interspecific pollen transfer

Plants that share generalist pollinators are more likely to be affected by some aspect of Interspecific Pollen Transfer (IPT) than plants that rely on one specialized pollinator (Waser 1978, Morales and Traveset 2008). Interspecific Pollen Transfer is a term used to describe a competitive effect occurring among plants for pollinators, specifically pollen movement by pollinators to different plant species (Morales and Traveset 2008). Interspecific Pollen Transfer may result in a decrease in reproduction rates of some plant species and it may occur through conspecific pollen loss or heterospecific pollen deposition (Waser 1978). Investigations into IPT effects are ongoing due to the difficulty in generalizing varied ecosystems interactions.

2.2.1 Conspecific pollen loss

Conspecific Pollen Loss (CPL) can occur when a pollinator visits several different plant species in a foraging session where pollen is unintentionally deposited onto other species' stigmas or floral parts (Waser 1978, Murcia and Feinsinger 1996, Morales and Traveset 2008). Plants require pollinators to deposit conspecific pollen to increase genetic variability in the plant species (Ellstrand 1992, Kwak et al. 1998); higher conspecific pollen deposition usually results in higher male fitness and larger seed outputs, which also results in high female fitness (Waites and Agren 2004, Morales and Traveset 2008). The area on a pollinator's body where pollen is deposited may determine the likelihood of CPL (Armbruster et al. 1994); if a pollinator visits several types of plant species but pollen from each plant species is gathered and deposited on a unique body part, then the impact of CPL may be decreased because the pollen deposited on each plant species' stigma will most likely be conspecific (Armbruster et al. 1994). Depending on the flight patterns of pollinators and the area pollen was deposited on a pollinator, a higher abundance of plant species visited during a foraging flight could increase CPL, affecting plant reproduction rates.

2.2.2 Heterospecific pollen deposition

Heterospecific pollen deposition (HPD)could result in a loss of surface area available for conspecific pollen, deemed "stigma clogging" (Galen and Gregory 1989, Morales and Traveset 2008). Pollen of several species deposited onto a stigma may in some cases clog a stigma; therefore, preventing pollen from a conspecific plant initiating pollen tube growth and reaching the ovule for seed production (Waser 1978). By reducing the available area receptive to conspecific pollen, stigma clogging may result in a decreased or absent seed set (Waser 1978).

HPD may facilitate pollen allelopathy, which is the release of pollen chemicals that reduce pollen germination and seed set on recipient stigmas and ovules (Sukhada and Jayachandra 1980, Murphy and Aarssen 1989). Allelopathic effects occur when chemicals on pollen from one plant species can alter reproduction mechanisms on a secondary species (Sukhada and Jayachandra 1980), such as reducing seed set (Murphy and Aarssen 1995b), reducing ovule development (Thomson et al. 1982), or reducing pollen tube growth (Sukhada and Jayachandra 1980). Pollen allelopathic effect is a less studied field within IPT as it is not a common occurrence (Murphy et al. 2009b) and it requires time and precision to isolate a variety of chemicals (Murphy et al. 2009a).

2.3 Effects of invasive species on pollination ecology

Urbanization and fragmentation can facilitate the establishment of exotic and invasive species (Gelbard and Belnap 2003, Lilley and Vellend 2009). Therefore, in addition to habitat loss, invasive species may also further exacerbate biodiversity loss. The terms invasive, alien, exotic, and non-indigenous are often used interchangeably to describe plants that are not native to a region. Invasive species are defined as organisms that have become established in an ecosystem where historical populations were never found, which tend to outcompete established native plant populations, threatening their sustainability and biodiversity (Richardson et al. 2000a, IUCN 2011). Conversely, exotic species are defined as a "species occurring outside of its natural range" and are not considered aggressive competitors against native species (IUCN 2000). Invasive species establishment is often a result of direct or indirect human introductions (IUCN 2000, Pyšek et al. 2004) and invasive species are able to become established due to their ability to outcompete native species for resources such as nutrients, light, space, and water

(Levine et al. 2003). Invasive species' abilities to outcompete natives can markedly alter an ecosystem, threatening the survival of native organisms and decreasing native biodiversity.

2.3.1 Negative effects of invasive species within ecosystems

Interspecific Pollen Transfer can occur in the absence of invasive species; however, the addition of invasive species may not only negatively affect an ecosystem through direct competition for resources but they may also increase the effect of IPT. Invasive species have the potential to affect current plant-pollinator relationships because they frequently have floral characteristics that are not specialized, allowing them to be readily integrated into the ecosystem by pollinators (Richardson et al. 2000a). Native pollinators have been observed to easily incorporate invasive species into their foraging efforts (Chittka and Schurkens 2001, Moragues and Traveset 2005, Bjerknes et al. 2007, Lopezaraiza–Mikel et al. 2007), usually because invasive species have similar floral shapes and colours to native congeners but at a higher floral density, thereby attracting pollinators but not requiring them to change their foraging behaviour (Memmott and Waser 2002, Bjerknes et al. 2007). When potted plants with both an invasive and native plant species were placed side by side, pollinators chose to visit the invasive plant species more frequently than native species (Grabas and Laverty 1999, Brown et al. 2002). The discrepancy between conclusions based on lab experiments and in situ experiments further demonstrates the need for in situ field experiments to continue to expand our knowledge of these complex plant-pollinator interactions.

Conspecific Pollen Loss may increase with the presence of invasive plant species due to the increased abundance of plant species that pollinators can forage on (Brown et al. 2002, Stout and Morales 2009) thereby potentially decreasing pollinator constancy and depositing pollen on improper species (Flanagan et al. 2009). Pollinators may increase their visitation to invasive

plant species and reduce visitation to native plant species, thereby reducing total conspecific pollen deposition (Brown and Mitchell 2001, Chittka and Schurkens 2001). Conspecific Pollen Loss may also be increased with the arrival of an invasive pollinator generalist due to their lack of established foraging preferences within the ecosystem (Grabas and Laverty 1999, Brown et al. 2002, Morales and Aizen 2006). However, invasive pollinators have not been well studied, except for *Apis mellifera* (Traveset and Richardson 2006), and their effect on the conspecific pollination patterns within meadow communities is generally unknown.

The integration of invasive plants into foraging routes of pollinators may result in increased deposition of heterospecific pollen, restricting conspecific pollen from creating pollen tubes and producing seeds. Some invasive species have been found to produce larger quantities of pollen than their native counterparts, for example *Lythrum salicaria* produced two times as much pollen per flower than *Lythrum alatum* (Brown and Mitchell 2001) and the potential to saturate stigmas with heterospecific pollen can be increased leading to a higher probability of stigma clogging. Additionally, a reduced seed set has been observed in hand pollination studies where invasive plant species pollen is deposited onto native plant species' stigmas within a laboratory setting (Brown and Mitchell 2001, Moragues and Traveset 2005).

Hand pollination experiments deposit a larger quantity of pollen on stigmas than recorded through field observations and therefore the same quantity of heterospecific pollen deposition has not been recorded on native plant stigmas through natural entomophilic pollination (pollination by insects) (Moragues and Traveset 2005, Larson et al. 2006, Bartomeus et al. 2008).

The establishment of one invasive species can facilitate the establishment of future invasive species, sometimes referred to as "Invasive Species Meltdown" (Simberloff and Von

Holle 1999). For example, the introduction of an invasive plant can aid in the future establishment of an invasive herbivore (Engelkes and Mills 2013). The presence or establishment of one invasive species may pose minor threats to the plant-pollinator community by itself; however, the potential for compounding problems from invasive species in the future can create cause for concern. Specifically, the establishment of invasive plants within meadow communities could aid in establishing future invasive plants or the establishment of invasive pollinators, which might further threaten biodiversity and plant-pollinator interactions.

2.3.2 Positive effects

Alternatively, invasive species might aid in reducing widespread pollinator declines. Pollinator populations could potentially increase if the increased competitive effect of invasive species produces higher floral abundance, greater nectar quantities, and larger pollen loads; thereby providing greater resources in general and/or resources during native plant floral display gaps to pollinators (Graves and Shapiro 2003, Tepedino et al. 2008). Additionally, pollinators may be attracted to meadows with invasive species, due to the high floral display, and upon arrival forage on native species due to historical preferences (McKinney and Goodell 2011). McKinney and Goodell (2011) reported an increased seed set on a native plant species in the presence of an invasive plant McKinney and Goodell (2011) due to higher visitation rates in invaded sites compared to not-invaded sites. Similarly, Moragues and Traveset (2005) determined a facilitative effect on pollinator visits to adjacent native plant species in the presence of an invasive plant species. By providing greater attraction to pollinators, invasive species might have a facilitative effect on pollinators if pollinators display floral constancy to native plant species once arriving at a meadow.

2.3.3 Absence of effect

Invasive species may also have little to no effect on the reproductive success of native species in terms of pollination, excluding their competition for water, nutrients, or light. Although there are fewer studies demonstrating a neutral effect of invasive species, Moragues and Traveset (2005) observed no effect of the invasive species *Carpobrotus* on visitation rates or seed set for the native species *Cistus monspeliensis*.

2.3.4 Future research on interspecific pollen transfer by invasive species

The impact of invasive plant species pollen on native plant species stigmas is increasingly being investigated (Brown and Mitchell 2001, Moragues and Traveset 2005, Bartomeus et al. 2008, Tepedino et al. 2008, Kandori et al. 2009, Dietzsch et al. 2011b, McKinney and Goodell 2011, Williams et al. 2011, King and Sargent 2012). Although many studies assessing the potential effect of invasive plant species on current ecosystems have focused on pairwise interactions (Bosch and Waser 1999, Grabas and Laverty 1999, Brown and Mitchell 2001, Chittka and Schurkens 2001, Brown et al. 2002, Cariveau and Norton 2009, Flanagan et al. 2009, Kandori et al. 2009, Gomez et al. 2010, McKinney and Goodell 2011), there are only a few community scale studies to date (Moragues and Traveset 2005, Larson et al. 2006, Bartomeus et al. 2008, Arceo-Gomez and Ashman 2011). Community level studies may provide a better coherent understanding than pairwise studies because complex species networks and linkages are assessed. One way of determining the community effect of an invasive plant species has been to create plant-pollinator webs, documenting the flow of pollen by pollinators and the visitation routes of pollinators throughout the plant community (Lopezaraiza–Mikel et al. 2007). Literature characterizing pollinator webs is limited and therefore the ability to further

interpret the potential effects of IPT within a community with or without an invasive species is sparse.

Although many studies attempt to generalize the effects of exotic or invasive species on one or several co-occurring native species, the complicated relationships between these groups has led to many conflicting result within the literature. Studies researching the effect of *Lythrum salicaria* have different conclusions on its' effect on native plant species. Moragues and Traveset (2005) reported a facilitative effect on pollinator visits to adjacent native plant species in the presence of an invasive plant species, whereas Brown et al. (2002) reported a negative effect on visitation rates. Studies have shown that invasive species cogeners usually have a negative effect on their native cogener (Brown et al. 2002, Kandori et al. 2009). This is most likely due to similar flower structures (Memmott and Waser 2002), which do not require foraging habit alterations by pollinators.

Additionally, studies have recently been conducted to determine if conflicting results may be due to different densities of invasive plant species present (Moeller 2004, Munoz and Cavieres 2008). Grabas and Laverty (1999) determined that the degree of negative effects on native species did differ between different densities of invasive plant species. Density-dependent studies may aid in clarifying conflicting results in the current literature but too few studies have been conducted to confirm whether various densities will in fact produce differing degrees of effects. Variations of impact from invasive species on native species between sites can occur in a similar region and between field seasons within the same study (Campbell and Motten 1985, Moragues and Traveset 2005, Larson et al. 2006). This suggests that generalizing invasive plant species effects on native plant species may not be recommended because effects may be both site

specific and species specific, but too few studies have been conducted to be able to accurately state that no generalizations can be made about the effects of invasive species.

There are too many factors: plant species, site location, pollinator species, temporal and spatial species abundance differences, and plant species' densities to accurately predict the exact effect invasive species will have on all meadow communities. Due to variable results from plant-pollinator studies that focus on invasive species, there is a need to determine current plant-pollinator interactions, regionally, prior to the establishment of invasive species to allow for a better prediction of their effects within specific systems. In the absence of monitoring programs prior to invasive species establishment, regional monitoring programs should be initiated to determine what effect invasive species have on current plant-pollinator interactions. Rapid establishment of monitoring programs would allow for better assessments of future meadow conservation efforts.

2.4 Declines in pollinators is a key reason for lower pollination success

Declines in pollinators can also potentially decrease pollination success. Recent sharp declines in bee pollinators, both wild and commercialized, have sparked investigations hypothesizing that pathogens, parasites, viruses, or pesticides are affecting bees and causing the recently termed "Colony Collapse Disorder" (vanEngelsdorp et al. 2009). No one single cause has been determined as the main trigger of colony collapses and bee losses; however, an increasing number of studies are being conducted to determine how and to what extent prevalent pathogens, viruses, and parasites hinder bee activity and to what extent the combination of these three types of stressors increases fatalities (Mayack and Naug 2010, Martin-Hernandez et al. 2011). Most pollinator decline research is concentrated on the European bee species *Apis*

mellifera, due to its importance in the agricultural industry (Gordon et al. 1998, Menz et al. 2011).

2.4.1 Varroa Mite

The *Varroa* mite *(Varroa destructor)* switched from its natural host *Apis ceranae* to *Apis mellifera*, when *A. mellifera* was translocated into areas where *A. ceranae* was endemic (Oldroyd 1999). Repeated *V. destructor* feeding on adult bee and brood hemolymph physically injures bees by: reducing their protein content, reducing wet and dry body weight, and interfering with organ development (Schneider and Drescher 1987, Bowen-Walker et al. 1999). Nordstrom et al. (1999) determined that within 2 years of mite infestation, with no treatment to reduce populations, 88 % of the colonies sampled in Sweden perished. The parasitic mite in conjunction with viruses contributes to morphological deformities (small body size, shortened abdomen, and deformed wings), which reduces vigor and longevity. *V. destructor* has been determined as a virus transmitter for both the Kashmir Bee Virus and the Deformed Wing Virus (Bowen-Walker et al. 1999, Chen et al. 2006), making the presence of the *V. destructor* more lethal than just the presence of a mite.

2.4.2.Viruses

There are at least 18 viruses that have been detected in honeybees (Allen and Ball 1996). In one of the few studies assessing mortalities due to specific viruses Tentcheva et al. (2004) found that 92 % of 36 voluntary apiaries in France were infected by several viruses. Deformed wing virus was detected in the largest number of apiaries (97 %), followed by sacbrood virus (86 %), acue bee paralysis (86 %), black queen cell virus (86 %), chronic bee paralysis virus (28 %), and Kashmir bee virus (17 %) (Tentcheva et al. 2004). *V. destructor* was also sampled in all

apiaries for virus transmission. All viruses noted above were present in varying quantities except for the black queen cell virus and the chronic bee paralysis virus confirming the effect *V. destructor* can have in disease transmission between *A. mellifera* individuals. The presence of viruses decreases bee fitness, making them more susceptible to other stressors (Bowen-Walker et al. 1999, Evans 2001).

2.4.3.Pesticides

In conjunction with exposure to parasites, pathogens, and viruses, insect pollinators are exposed to chemical pesticides if their nesting sites are near or within agricultural fields (Stokstad 2007). The exact effects of chemical pesticide exposure varies between taxa (Thompson and Hunt 1999); however exposure may make contracting viruses and pathogens easier due to a decrease in overall fitness as a result of pesticide exposure. Vidau et al. (2011) determined that exposure to fipronil or thiacloprid (pesticides) in conjunction with an infection by *Nosema ceranae* (a parasite that has been shown to alter bees' foraging habits) led to higher mortality rates in *A. mellifera*. The high probability for pollinators in southern Ontario to come into contact with pesticides because of the mixed matrix land use surrounding natural areas is troublesome due to the potential cascading negative effects on pollinator longevity.

2.4.4 Pathogen spillover

Pollinators reared for commercial pollination are hypothesized as a vector of pathogens, viruses, and parasite transference to native pollinators, known as "pathogen spillover" (Daszak et al. 2000, Power and Mitchell 2004). Reviewing Canadian *Bombus* species data Colla et al. (2006) determined that commercialized *Bombus* species had a higher rate of parasite infection than wild *Bombus* species. Colla et al. (2006) also discovered increasing infection rates of wild pollinators caught near greenhouses housing commercialized bees compared to areas at a

distance from greenhouses in southern Ontario. Pathogen spillover is a relatively new concept for pollinator studies but it is becoming important as commercialized *A. mellifera* and *Bombus* species are increasingly used for crop pollination.

2.5 Meadow conservation in Ontario

Federal, provincial, and local non-profit organizations all contribute to conserve biodiversity in Ontario (Kanter 2005). Federally, Environment Canada creates and manages National Wildlife Areas and Migratory Bird Sanctuaries, while Parks Canada creates and manages National Parks and National Marine Conservation Areas (Environment Canada 2014). The Ministry of Natural Resources manages Ontario Parks, crown land, and fish and wildlife resources (Ministry of Natural Resources 2011). In addition, the Ministry of Natural Resources conducts research, as well as, manages and monitors endangered species (Ministry of Natural Resources 2011). Conservation Authorities manage and maintain natural resources on a watershed basis (Conservation Ontario 2009). A total of 3.5 % area of the Mixedwood Plains ecozone, which encompasses both the Eastern Deciduous Forest and the Great Lakes St. Lawrence region, is considered protected (Ontario Biodiversity Council 2010). Over half of these protected areas are held by Conservation lands (Conservation Authorities and Land Trusts) and the remaining held by regulated protected areas (Provincial Parks, National Parks, Wilderness Reserves) (Ontario Biodiversity Council 2010). Although the Ministry of Natural Resources and Environment Canada are large conservation bodies, Conservation Authorities are often responsible for implementing conservation measures (Environmental Commissioner of Ontario 2012).

Conservation Authorities were established in 1946 by the Ontario Provincial Government through the Conservation Authorities Act (1946) to regulate and protect Ontarians from flooding and erosion. There are currently 36 Conservation Authorities in Ontario (Conservation Ontario 2009). Since the establishment of the Conservation Authorities Act (1946) the mandate of Conservation Authorities has broadened to include all natural resources except for "gas, oil, coal and minerals"; the mandate now includes the "protect[ion], manage[ment] and restor[ation] [of] Ontario's woodlands, wetlands and natural habitat" (Conservation Ontario 2009).

In order to fulfill their mandate, Conservation Authorities gain their funding through multiple sources. On average, the largest source of funding is obtained through self-generated revenue from visitors and donations (42%) and the remaining through municipal (33%), provincial (23%) and federal (2%) levies or grants (Conservation Ontario 2009). Due to the reliance on self-generated revenue, goals and resources to carry out protective measures against biodiversity loss varies amongst Conservation Authorities (Kanter 2005). Conservation Authorities must balance conservation and monitoring plans with creating and maintaining recreational opportunities for visitors to ensure future revenue. Due to increased monetary restrictions future monitoring plans should be cost effective, easy to implement, and if possible created with a specific organization in mind to increase the likelihood of continuance (Fancy and Bennetts 2012).

3.0 Materials and methods

A combination of field sampling and laboratory methods were utilized to determine the quantity and type of pollen deposited on stigmas within southern Ontario meadows. General site conditions were gathered including percent cover, wind, and temperature. Stigmas and stamens were gathered from available forbs to evaluate pollen quantities and plant origin utilizing a compound microscope and pollen dying agents. The resulting data was statistically analyzed to determine if any plant(s) deposit numerous pollen grains on any specific species, thereby potentially reducing native plant reproduction.

3.1 Study sites

Study sites were located in Ontario, north of the Greater Toronto Area within two Conservation Authorities: the Credit Valley Conservation Authority (CVC) and the Toronto Region Conservation Authority (TRCA) (Figure 1). The average temperature is 8.2 °C with a minimum average temperature of -31.3 °C in January and a maximum average temperature of 38.3 °C in August (Environment Canada 2013). The average annual precipitation is 785.9mm (87 % rainfall) (Environment Canada 2013). Study sites were chosen based on initial visual vegetation inspection; ensuring areas were comprised of uncut graminoids and flowering forbs, as well as communication with Conservation Authority personnel. Only sites 0.5 ha or greater were considered to allow for adequate space for transects and data collection.

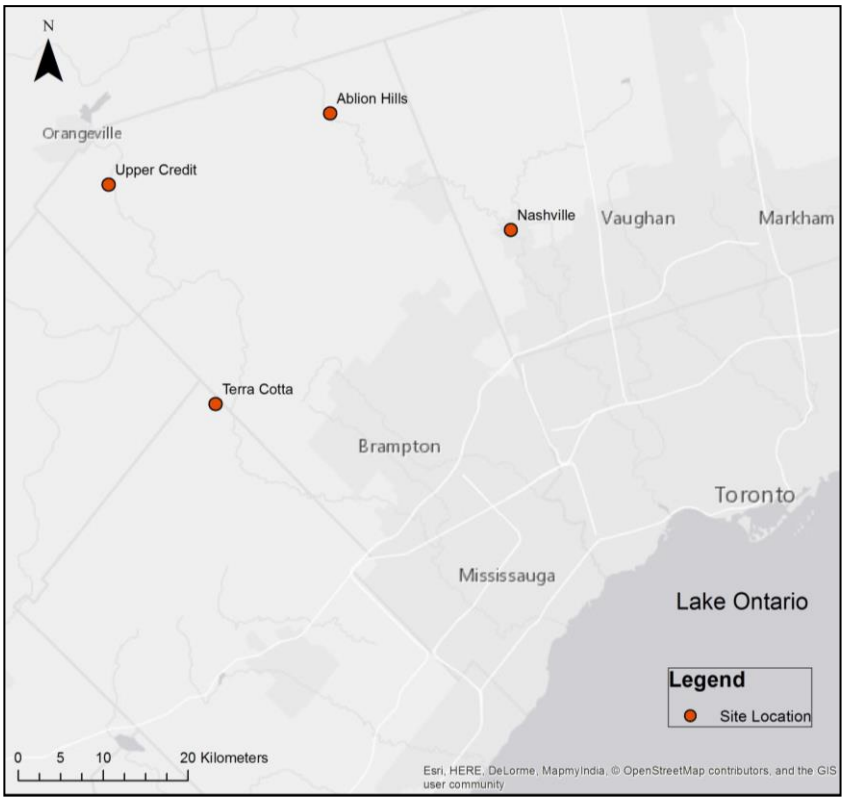

Figure 1 Study Site Locations

Terra Cotta Conservation Area is located within CVC (43°42'59.03"N, 079°56'59.77"W). The meadow studied within Terra Cotta is an open field next to a picnic shelter, away from regular trail use (Figure 2). It borders a deciduous forest and a picnic area**.** Terra Cotta was a recreational destination, with 137 campsites, hundreds of picnic tables and a large swimming pool (Credit Valley Conservation Authority 2008). During the 1980's, a shift towards natural heritage conservation and environmental protection occurred. By the mid 1990's the campsites, swimming pool,

Figure 2 Terra Cotta Study Site, Terra Cotta Conservation Area

and subsequent recreational facilities were removed and replaced with natural areas and a wetland left to be unmanaged (Credit Valley Conservation Authority 2008). In 2004, CVC created an initial Management Plan for Terra Cotta and every five years their management plan is reviewed and revised.

Upper Credit Conservation Area is located within CVC (43°53’23.73”N, 080°03’41.93”W). The meadow borders an old farmer’s field and a trail adjacent to a mixed deciduous and coniferous forest (Figure 3). The trail is well used as it is near the beginning of the trail complex. There is an extensive trail winding through meadow and forested habitat. Upper Credit was previously agricultural and livestock use (Credit Valley Conservation Authority 2013). It is a more recent purchase and extensive work has been done to improve the cold-water river running through the property.

Figure 3 Upper Credit Conservation Area

Figure 4 Albion Hills Conservation Area

Albion Hills Conservation Area is located within TRCA (43°56’01.58”N, 079°49’15.62”W). It borders a deciduous

forest and slopes down towards a wet meadow (Figure 4). Half way through the field season, a new trail was created through the area; otherwise, it was secluded and not well visited. Albion Hills was previously agricultural fields, as well as a man-made swimming lake called Albion Lake (Toronto and Region Conservation Authority 2013b). It currently has both conservation areas, as well as recreational areas with trails for dog walking and birding intermixed with serviced and un-serviced campsites, swimming pools, and picnic areas (Toronto and Region Conservation Authority 2013a).

Nashville is a tract of land under TRCA jurisdiction (43°50’52.74”N, 079°38’31.34”W). It is not considered a formal Conservation Area as it was recently acquired but it is open for public use. It borders a trail adjacent to a riparian area along the Humber River (Figure 5). The meadow is located opposite a privately owned mowed area. The Nashville Resource Management Tract includes approximately 700 hectares, most of which is adjacent to the Humber River near Bolton and Kleinburg (Toronto and Region Conservation Authority 2013c). It is a mix of forest, shrub land, meadow, and riparian areas. Currently, Nashville is under review and a management plan is being created with input from several stakeholders (Toronto and Region Conservation Authority 2013c).

Figure 5 Nashville Management Tract

3.2 Field study

Field work was conducted from mid-June through September 2011 during the growing season. Sampling was not performed in May due to the difficulty of obtaining collection permits, as well as locating non-mowed sites within Conservation Authorities.

3.2.1 Pollinator observations

Within each site, four transects spanning the width/length of each meadow were established (maximum length=60 m) (Wikstroem et al. 2009). Quadrats 1 m x 1 m (Larson et al. 2006, Fang and Shuang-Quan 2013) were placed randomly on the left or right side of each transect, every 10 m (maximum number of quadrats=6). Transects were created with a gap greater than 5 m apart, thereby minimizing sampling bias. Within each quadrat, a 15 minute observation period of pollinator interactions was conducted to determine pollinator visitation (Popic et al. 2013). All pollinators landing on flowers (within the quadrat) were recorded for all 15 minutes (Popic et al. 2013). All flower species from each pollinator visited were recorded. Each quadrat was observed between 0930-1900 (hours of operation for Conservation Areas) during sunny days with temperatures above 14°C (Williams 2011, Rader et al. 2012) with wind speeds less than 4.5 ms^{-1} or a 3 on the Beaufort scale (Larson et al. 2006, Williams 2011). Quadrats were sampled once per visit to avoid undue disturbance to vegetation. Due to weather and restricted access sampling occasionally occurred at one site over multiple days. Each site was sampled at least 5 times. A Kestrel 4000 Pocket Weather Tracker was used at the beginning of each transect walk to ensure low wind speeds (<5 ms^{-1}) throughout each transect walk (Rader et al. 2012). Effort was made to sample each site within the same week to ensure consistency. Visual confirmation of pollinators touching reproductive parts was difficult therefore pollinators were counted and recorded if they landed on a flower (Lopezaraiza–Mikel et al. 2007, Fang and

Shuang-Quan 2013). In addition, each floral species visited by the pollinator was recorded. Due to time limitations and monetary restrictions a general percent plant cover within each quadrat was recorded. The number and size of the quadrats were chosen to allow for visual observation of pollinators, as well as, the ability to complete all transects within a site in one day to reduce the effect of environmental variables. The direction of transect walk was randomly determined at the start of each day to reduce sampling bias.

3.2.2. Stigma collection and analysis

Stigmas were harvested from 227 (55-63 per site) flowering herbaceous plants within the designated site boundaries throughout the study period. Grasses were excluded, due to the anemophilous (wind pollinated) nature of grasses as opposed to the entomophilous nature of many wildflower species. Stigmas were chosen from flowering plants in between observational transects. Following Kearns & Inouye (1993) stigmas were placed within a solution of 75 % ethanol and distilled water in a 20x150 mm glass test tube until lab analysis could be performed. The test tube vials were subsequently covered with parafilm to prevent evaporation of the solution and to prevent pollen from entering vials through wind transfer (Murphy 1992). Forceps were cleaned with 90 % ethanol in between each stigma collection to avoid pollen contamination. Stigma collection was completed between June-September 2011, thereby gaining a representative sample of the summer flowering season. Stigmas were harvested at each site during every visit. The number of stigmas harvested depended on the estimated visual percent of coverage per plant species, as well as, the time of year, in accordance with both Conservation Authorities' permit policies. A reference pollen collection was created during field work at each site (Lopezaraiza–Mikel et al. 2007, Fang and Shuang-Quan 2013, Popic et al. 2013). Four stamens of each flowering plant were collected from plants within the site, around the site, and

along trails adjacent to the site (up to 10 m). Stamens were preserved in the same fashion as stigmas.

In the lab, stigmas were placed on a slide and were heated on a hot plate with Glycerin Jelly Stain and Crystalline basic Fuchsin tint (Kearns and Inouye 1993, Lopezaraiza–Mikel et al. 2007, Rader et al. 2012, Fang and Shuang-Quan 2013). A cover slip was placed onto stigmas once the stain was warm and liquid to be analyzed under the microscope (Kearns and Inouye 1993, Lopezaraiza–Mikel et al. 2007, Rader et al. 2012, Fang and Shuang-Quan 2013). The same method was applied to stamens to create the pollen reference collection.

Stigmas and stamens were analyzed under a 400x magnification compound microscope (Leica DM750)(Fang and Shuang-Quan 2013). Variation in pollen shapes (i.e. circular, oblong etc..), sizes, pores, and spines were utilized to identify individual pollen species (Kapp 1969) based on the reference collection. Photographs of pollen on stamens were used for the reference collection and pollen on stigmas were counted and identified to the species level if possible.

3.3 Data analysis

The statistical analysis was performed in R 2.15.1 statistical software. A variety of techniques have been used in Interspecific Pollen Transfer studies with no one measurement consistently chosen (McLernon et al. 1996, Murcia and Feinsinger 1996, Larson et al. 2006, Montgomery and Rathcke 2012, Fang and Shuang-Quan 2013). A Shannon Diversity index (H`) was used to determine the diversity of pollen deposition on collected stigmas in the Vegan package. The Shannon-Wiener diversity index is one of the most widely used diversity indices within conservation (Lande 1996, Jost 2006). Diversity indices are not direct measures of diversity but rather a measurement of equitability/uncertainty (Whittaker 1972); however, they can provide valuable information on how systems differ in their species composition (Jost 2006).

Each diversity index calculates equitability differently and therefore has different assumptions and weaknesses (Whittaker 1972, Lande 1996). The Shannon Diversity index is described as the probability that two entities chosen at random will in fact be the same (McCune and Grace 2002). It can have biases when small sample sizes are involved (Lande 1996) and affected by the quantities of species within the middle of the sequence rather than rare or abundant species (Whittaker 1972). Determining the Shannon diversity value of pollen deposition will aid in identifying stigma species that receive pollen from a variety of sources thereby informing plant visitation patterns to individual species. The Shannon Diversity Index was utilized instead of species richness because it provides information about the quantity of pollen grains, which is important for understanding the potential negative effect of HPD, as an increase in pollen quantity from multiple species can potentially negatively affect reproduction in contrast to small pollen quantities from multiple species. Evenness (E) was calculated to determine the relative abundance of deposited pollen grain species. Evenness values aid in highlighting the depositional nature of pollen, where it can provide insight on number of species that represent the majority of pollen deposited on stigmas, either 1 or 2 species comprising the majority of pollen deposited or dispersed evenly throughout many species. A one-way ANOVA comparing the variance between nativity/species (independent) and Shannon Diversities (dependent) was performed for species with more than 1 occurrence (Lande 1996, Awal and Svozil 2010). To further elucidate the variation in Shannon Diversities between native and invasive species, a species specific ANOVA was performed with a post-hoc Tukey's test. Total pollen counts per stigma species were also graphed for species that demonstrated a higher Shannon Diversity value and statistically significant diversity indices, thereby highlighting its heterospecific pollen donor species.

Counts of pollinators observed touching a flower were summed over the entire sampling period for each site and their abundance calculated. These abundances were standardized by the number of minutes observed per site to account for differences in total sampling time between sites. Sampling times varied between sites due to quadrat lengths differences, weather that prevented sampling, and restricted access to sites by Conservation Authorities during community events.

4.0 Results

A total of 22 plant species were observed where the 5 taxa species found in Table 1 represent 70-90 % of the observed percent cover and the remaining 10-30 % of the observed plant cover was distributed amoung 10 to 22 species depending on site (Table 2). Typically the top 3 species consisted of *Solidago/Oligoneuron* species, *Aster/ Symphyotrichum* species, and Gramanoid species with inclusions of *Vicia cracca* and *Daucus carota*.

Table 1 Percent Cover observed at all sites for the highest 3 species

Site	Scientific Species Names	Average Observed Percent Cover
Albion Hills	*Solidago/Oligoneuron* spp.	62
	Vicia cracca L.	15
	Daucus carota L.	15
Nashville	Gramanoid spp.	38
	Solidago/Oligoneuron spp.	22
	Vicia cracca L.	20
Terra Cotta	*Aster/ Symphyotrichum* spp.	27
	Solidago/Oligoneuron spp.	26
	Gramanoid spp.	17
Upper Credit	*Solidago/Oligoneuron* spp.	47
	Gramanoid spp.	20
	Aster/ Symphyotrichum spp.	16

4.1 Vegetation species observations

Seven native plant species were observed within the sites' boundaries out of a total of 22 species, with the remaining fifteen species comprised of exotics (Table 2). General observations of plants located outside site boundaries were made to ensure their stamens were collected since pollinators may have visited them prior to travelling within site boundaries. The observed species surrounding site boundaries were composed of three natives and eight exotic species (Table 3).

Similar to Popic et al. (2013) and Forup and Memmott (2005) *Aster/Symphyotrichum* species and *Solidago/ Oligoneuron*, as well as *Cirsium* species were grouped together due to indiscernible differences in pollen shape underneath a microscope. Terra Cotta had the largest number of stigma species collected (14) compared to Upper Credit (9), Nashville (9), and Albion Hills (8).

Aster/Symphyotrichum species (Asters), *Hypericum perforatum* (common St. John's Wort*), Solidago/ Oligoneuron spp*. (Goldenrods) and *Vicia cracca* (Cow Vetch) were found and sampled at all four sites, while, *Daucus carota* (Queen Anne's Lace) was found within all sites excluding Upper Credit (Table 2). There were 14 species sampled that were only found at one site, representing 64 % of the total species sampled.

4.2 Pollen diversity of stigma species

Typically there were <7 distinct deposited pollen species per plant species stigmas, except for Hypericum perforatum that had 13, across all four sites. There was little evidence for heterospecific pollen deposition in 92 % of the species sampled. The remaining 8 % that did exhibit heterospecific pollen deposition were exotic species. Pollen density ranged from 5.6 pollen grains per stigma (*Clinopodium vulgare L.* at Upper Credit) to 1003.8 pollen grains per stigma (*Ranunculus acris L.* at Upper Credit) (Table 4). Upper Credit displayed the highest average pollen density and Albion Hills displayed the lowest average density (Table 4).

An analysis of variance (ANOVA) on exotic and native species' averaged Shannon Diversity values was not considered significantly different ($F=3.356$, d.f=1,6, $P=0.117$). When analyzed separately by species a significant difference was determined ($F=8.021$, d.f=5,14, $P=0.000941$). A post hoc Tukey test highlighted that pollen diversity found on 5 plant species differed significantly at an alpha of $p<0.05$. The diversity of pollen found on *Aster* stigmas was significantly lower than pollen found on *Hypericum* or *Cirsium* stigmas. In addition, pollen

found on *Cirsium* species was significantly higher than *Daucus* or *Solidago*, and pollen found on *Solidago* was significantly lower than *Hypericum*. All other pairwise interactions of Shannon Diversity indices were deemed not significantly different.

Table 2 Species observed within sites: Terra Cotta (TC), Upper Credit (UC), Albion Hills (AH), and Nashville (NASH). * Scientific names obtained from http://www.uoguelph.ca/foibis/

Common Name	Scientific Name*	Status	Location found
Grooved Agrimony	*Agrimonia striata* Michx.	Native	TC
Aster species	*Aster/ Symphyotrichum spp.*	Native	AH, NASH, TC, UC
Wild Basil	*Clinopodium vulgare* L.	Native	TC, UC
Spotted Joe Pyeweed	*Eutrochium maculatum* L. E.E. Lamont	Native	UC
Common Evening Primrose	*Oenothera biennis* L.	Native	UC
Black-eyed Susan	*Rudbeckia hirta* L. *var. hirta*	Native	NASH
Goldenrod species	*Solidago/Oligoneuron spp.*	Native	AH, NASH, TC, UC
Common Yarrow	*Achillea millefolium* L.	Exotic	TC
Brown Knapweed	*Centaurea jacea* L.	Exotic	TC
Thistle spp.	*Cirsium spp.*	Exotic	TC, NASH, UC
Smooth Hawk's Beard	*Crepis capillaris* (L.) Wallr.	Exotic	TC
Queen Anne's Lace	*Daucus carota* L.	Exotic	AH, NASH, TC
Deptford Pink	*Dianthus armeria* L.	Exotic	TC
Wild Teasel	*Dipsacus fullonum* L.	Exotic	TC
Viper's Bugloss	*Echium vulgare* L.	Exotic	AH
Common St. John's Wort	*Hypericum perforatum* L.	Exotic	AH, NASH, TC, UC
Butter-and-eggs	*Linaria vulgaris* Miller	Exotic	NASH
Bird's-foot Trefoil	*Lotus corniculatus* L.	Exotic	AH
White Sweet-clover	*Melilotus albus* Medik	Exotic	AH
Sulphur Cinquefoil	*Potentilla recta* L.	Exotic	TC
Meadow Buttercup	*Ranunculus acris* L.	Exotic	NASH, UC
Cow Vetch	*Vicia cracca* L.	Exotic	AH, NASH, TC, UC

Table 3: Incidental species observed at a site <10m from the boundaries of the study

Common Species	Scientific Name	Status
Canada Anemone	*Anemone canadensis* L.	Native
Common Milkweed	*Asclepias syriaca* L.	Native
Canada Fleabane	*Erigeron canadensis* L.	Native
Chicory	*Cichorium intybus* L.	Exotic
Field Bindweed	*Convolvulus arvensis* L.	Exotic
Oxeye Daisy	*Leucanthemum vulgare (Vaill.) Lam.*	Exotic
Black Medick	*Medicago lupulina* L.	Exotic
Curled Dock	*Rumex crispus* L.	Exotic
Bladder Campion	*Silene cucubalus* (Moench) Garcke	Exotic
Goat's-beard	*Tragopogon dubius* Scop.	Exotic
White Clover	*Trifolium repens* L.	Exotic

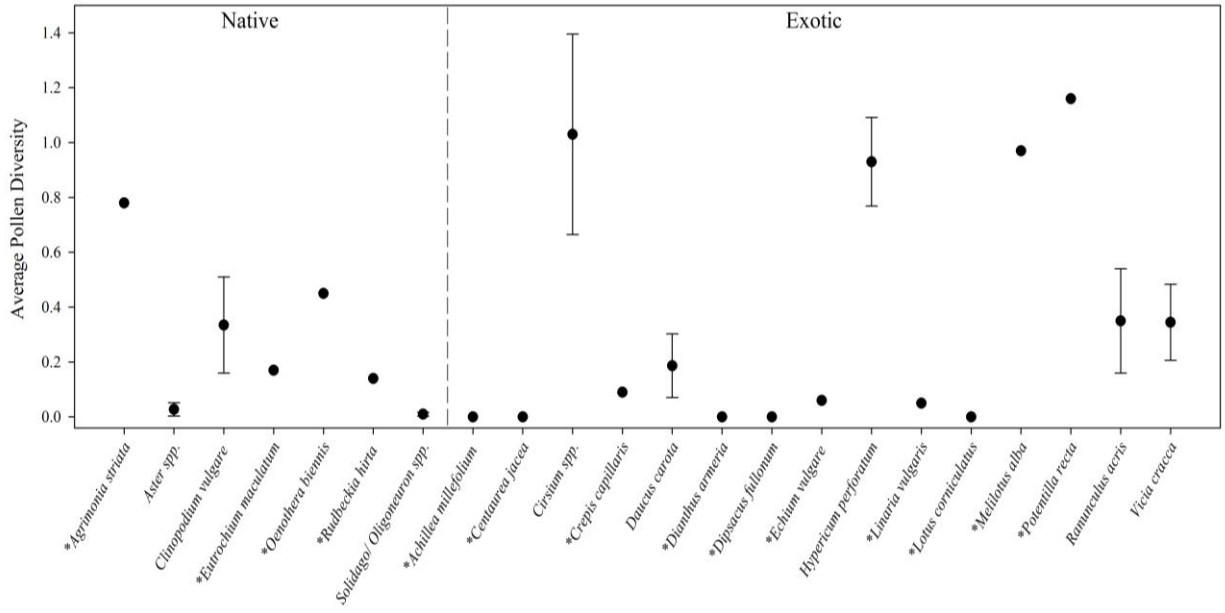

Figure 6 Average pollen diversity (H') separated by native and exotic species. Error bars represent standard error. n=227. *Indicates species only found at one site.

4.2.1 Native

Asters, Eupatorium perforatum, Oenothera biennis, Rudbeckia hirta, Clinopodium vulgare, and *Solidago*/*Oligoneuron* displayed little heterospecific pollen deposition (Table 5 and Figure 6). The few heterospecific pollen grains that were deposited were from a variety of species and did not originate from any one dominating species and were typically from exotic species. Due to similarities in *Aster/Symphyotrichum* and *Solidago*/*Oligoneuron* pollen grains, determining species origins and thereby HTP on these stigmas may be underestimated. *Aster/Symphyotrichum species, Clinopodium vulgare, and Solidago/Oligoneuron* species were the only native species found at more than one site.

Table 4 Pollen density (#grains/stigma) for species at all site locations. n=227.

Species Names	Site Locations			
	Albion Hills	Nashville	Terra Cotta	Upper Credit
Mean Pollen Density	49.4	130.0	69.0	246.4
Agrimonia striata Michx.			53.0	
Aster/ Symphyotrichum	39.8	49.7	52.8	69.9
Clinopodium vulgare L.			33.0	5.6
Eutrochium maculatum L. E.E.Lamont				140.8
Oenothera biennis L.				485.4
Rudbeckia hirta L. var. hirta		24.6		
Solidago/Oligoneuron	41.9	52.3	47.1	127.2
Achillea millefolium L.			6.5	
Centaurea jacea L.			44.7	
Cirsium spp.		22.0	20.7	9.3
Crepis capillaris (L.) Wallr.			213.0	
Daucus carota L.	24.5	11.9	33.3	
Dianthus armeria L.			49.7	
Dipsacus fullonum L.			15.6	
Echium vulgare	147.5			
Hypericum perforatum L.	43.0	136.9	74.7	229.0
Linaria vulgaris Miller		410.7		
Lotus corniculatus L.	36.0			
Melilotus albus Medik	20.3			
Potentilla recta L.			283.0	
Ranunculus acris L.		209.0		1003.8
Vicia cracca L.	42.0	252.9	39.3	147.0

Table 5 : Shannon Diversity index for all sites: Terra Cotta (TC), Upper Credit (UC), Albion Hills (AH), and Nashville (NASH). H' represents the Shannon Diversity Index. E represents Pielou's Evenness Index. n=227.

Stigma Species	AH		NASH		TC		UC		Average H' ± SE
	H'	E	H'	E	H'	E	H'	E	H'
Agrimonia striata					0.78	0.56			0.78
Aster/ Symphyotrichum spp.	0.10	0.09	0.01	0.01	0.00	0.00	0.00	0.00	0.04 ± 0.02
Clinopodium vulgare					0.51	0.74	0.16	0.23	0.34 ± 0.12
Eutrochium maculatum							0.17	0.16	0.17
Oenothera biennis							0.45	0.33	0.45
Rudbeckia hirta			0.14	0.13					0.14
Solidago/ Oligoneuron spp.	0.01	0.02	0.00	0.00	0.00	0.00	0.03	0.05	0.01 ± 0.01
Achillea millefolium					0.00	0.00			0.00
Centaurea jacea					0.00	0.00			0.00
Cirsium spp.			1.76	0.98	0.68	0.62	0.65	0.94	1.03 ± 0.30
Crepis capillaris					0.09	0.14			0.09
Daucus carota	0.00	0.00	0.40	0.21	0.16	0.15			0.19 ± 0.09
Dianthus armeria					0.00	0.00			0.00
Dipsacus fullonum					0.00	0.00			0.00
Echium vulgare	0.06	0.06							0.06
Hypericum perforatum	0.59	0.37	1.30	0.62	1.09	0.44	0.74	0.33	0.93 ± 0.14
Linaria vulgaris	-		0.05	0.05					0.05
Lotus corniculatus	0.00	0.00							0.00
Melilotus alba	0.97	0.70							0.97
Potentilla recta					1.16	0.72			1.16
Ranunculus acris			0.54	0.49	-		0.16	0.08	0.35 ± 0.13
Vicia cracca	0.44	0.32	0.68	0.38	0.04	0.04	0.22	0.20	0.35 ± 0.12

Evenness values range from 0-1 with higher values representing similar pollen quantity contribution from all donors.
Shannon diversity values range from 0-3.5 where the higher the value the greater number of pollen species present on a stigma.

4.2.2 Exotic

Achillea millefolium, Agrimonia striata, Centaurea jacea, Daucus carota, Dianthus armeria, Dipsacus fullonum, Lotus corniculatus, Ranunculus acris, and *Vicia cracca* were typically deposited with conspecific pollen and with little heterospecific pollen deposition (Table 5 and Figure 6). Similar to native stigmas, the heterospecific pollen originated from a variety of exotic species (data not shown).

Cirsium species and *Hypericum perforatum* had significantly higher Shannon Diversity values than *Aster* and *Solidago* species; furthermore, *Cirsium* was significantly higher than *Daucus* (Figure 6). Both *Cirsium species* and *Hypericum perforatum* exhibited a Shannon Diversity value greater than 1. *Potentilla recta* and *Melilotus alba* both had Shannon Diversity values close to or above 1 (1.16 and 0.97, respectively); however, they did not occur at multiple sites and therefore further statistical analysis could not be performed. An in-depth look at pollen species deposited on their stigmas will provide insight into donor species origin and highlight any commonly deposited pollen species (exotic or native) or patterns that can aid in explaining why these species exhibited higher Shannon Diversity values.

Cirsium (vulgare and arvense)

Cirsium species stigmas had the highest pollen diversity value in this study with a high corresponding evenness value at Nashville (Table 5). The pollen distributed on all *Cirsium* stigmas was comprised of four pollen species: *Cirsium* species, *Hypericum perforatum*, *Potentilla recta*, and *Vicia cracca* (Figure 7). *Cirsium* pollen represented the majority of pollen species deposited on *Cirsium* stigmas at Terra Cotta and was highlighted with a large evenness value; however, at Upper Credit and Nashville higher quantities of *Vicia cracca* pollen and

Hypericum perforatum (Figure 8), respectively, were observed. Pollen depositional patterns varied across sites for *Cirsium* species. Cirsium was one of the few species (see above) that displayed significantly different Shannon diversity indices with 3 different species (*Aster/ Symphyotrichum*, *Daucus carota*, and *Solidago/ Oligoneuron*).

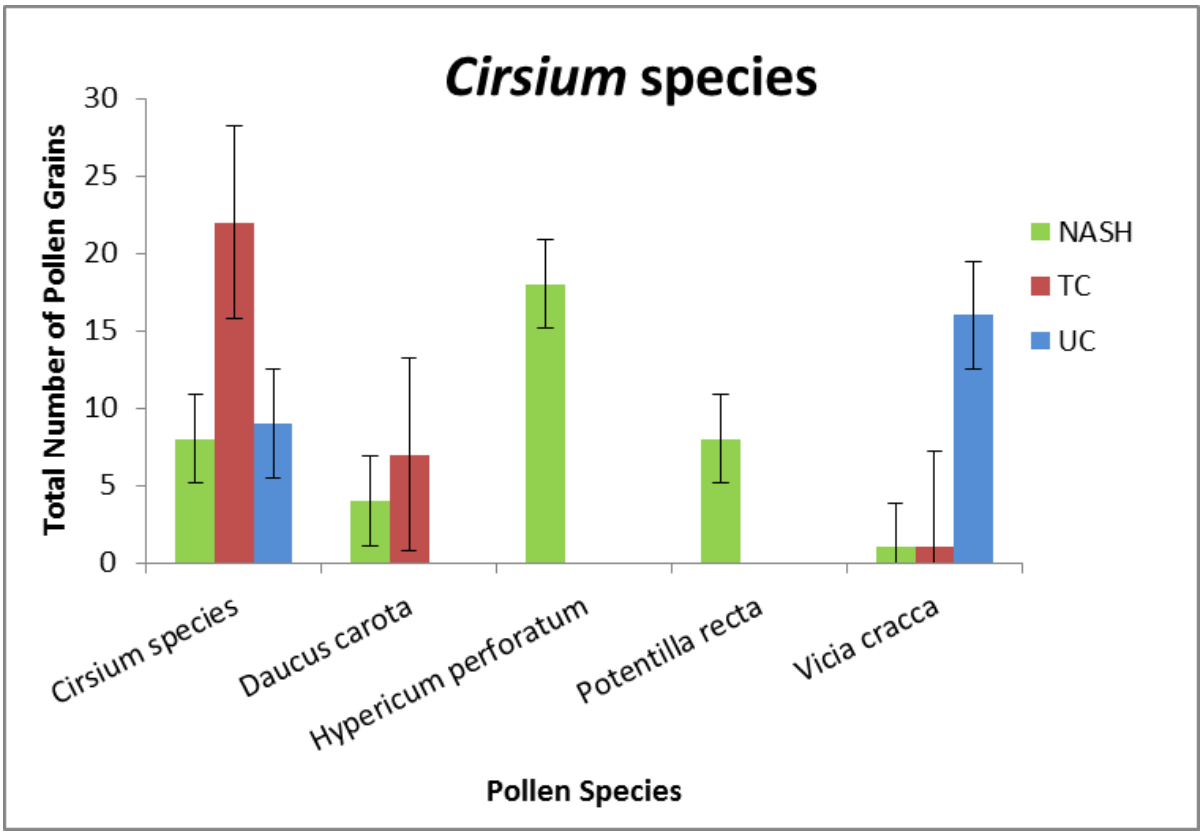

Figure 7 Total number of pollen grains identified on *Cirsium arvense* and *Cirsium vulgare* stigmas at Nashville (n=8), Terra Cotta (n=6), and Upper Credit (n=4). Error bars represent standard error

Hypericum perforatum

Hypericum perforatum was the only species to have a diversity value greater than 1.0 at two sites (Nashville and Terra Cotta) with evenness values in the low to mid-range suggesting several evenly deposited pollen species (Table 5). *Hypericum perforatum* also produced two pairwise Shannon Diversity values that were considered significantly different (see above) with *Aster/ Symphyotrichum* and *Solidago/ Oligoneuron*. In all four sites conspecific pollen was the majority of pollen deposited on *Hypericum perforatum* stigmas (Figure 8); however, *Hypericum perforatum* stigmas had the highest quantity of pollen species found on stigmas in this study. Nashville's corresponding evenness value was the highest amongst all four sites representing a large deposition of conspecific pollen with an even deposition of the remaining heterospecific pollen species (Figure 8).

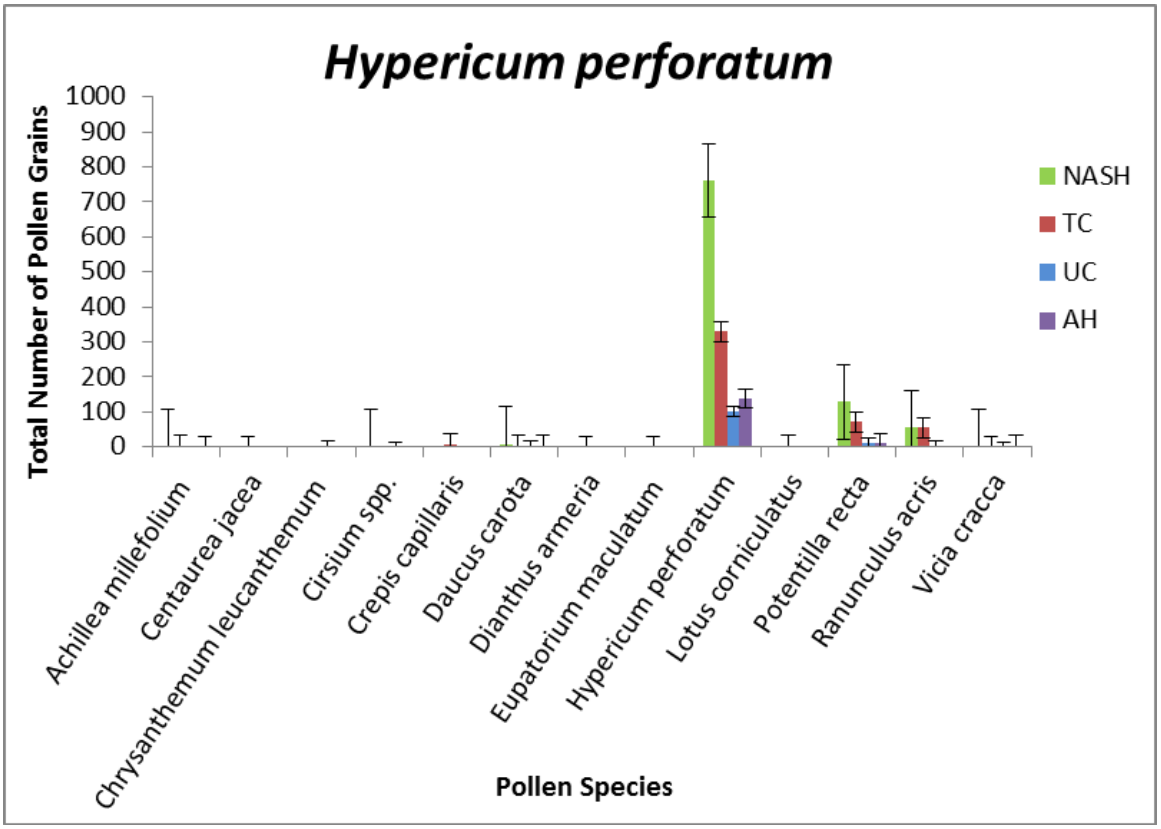

Figure 8 Total number of pollen grains identified on *Hypericum perforatum* stigmas at Nashville (n=10), Terra Cotta (n=7), Albion Hills (n=5), and Upper Credit (n=6). Error bars represent standard error

Melilotus alba

Melilotus alba was found within Albion Hills and had a diversity value slightly below 1 (0.97) and a high evenness value (0.70) within this study (Table 5). The evenness value can be attributed to the dominance of two main pollen species, *Melilotus alba* (Figure 9) and *Agrimonia striata* (Figure 10) with a small number of *Aster/Solidago/Oligoneuron* and *Daucus carota* pollen grains. Although both *Melilotus alba* (conspecific) and *Agrimonia striata* (heterospecific) pollen have similar depositional quantities the same pattern is not viewed on *Agrimonia striata* stigmas. *Agrimonia striata* stigmas sampled at Terra Cotta were deposited by conspecific pollen and secondly by *Dianthus armeria* (Figure 10).

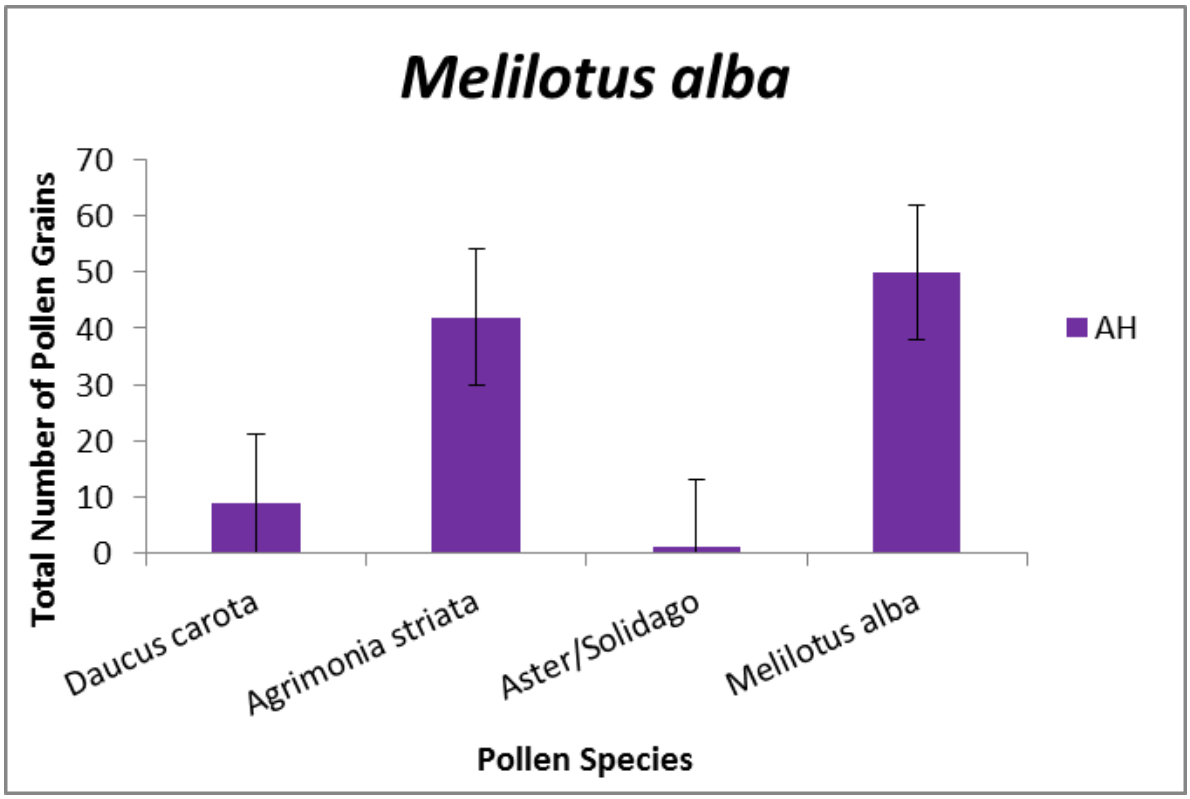

Figure 9 Total quantity of pollen grains identified on *Melilotus alba* stigmas at Albion Hills (n=8). Error bars represent standard error.

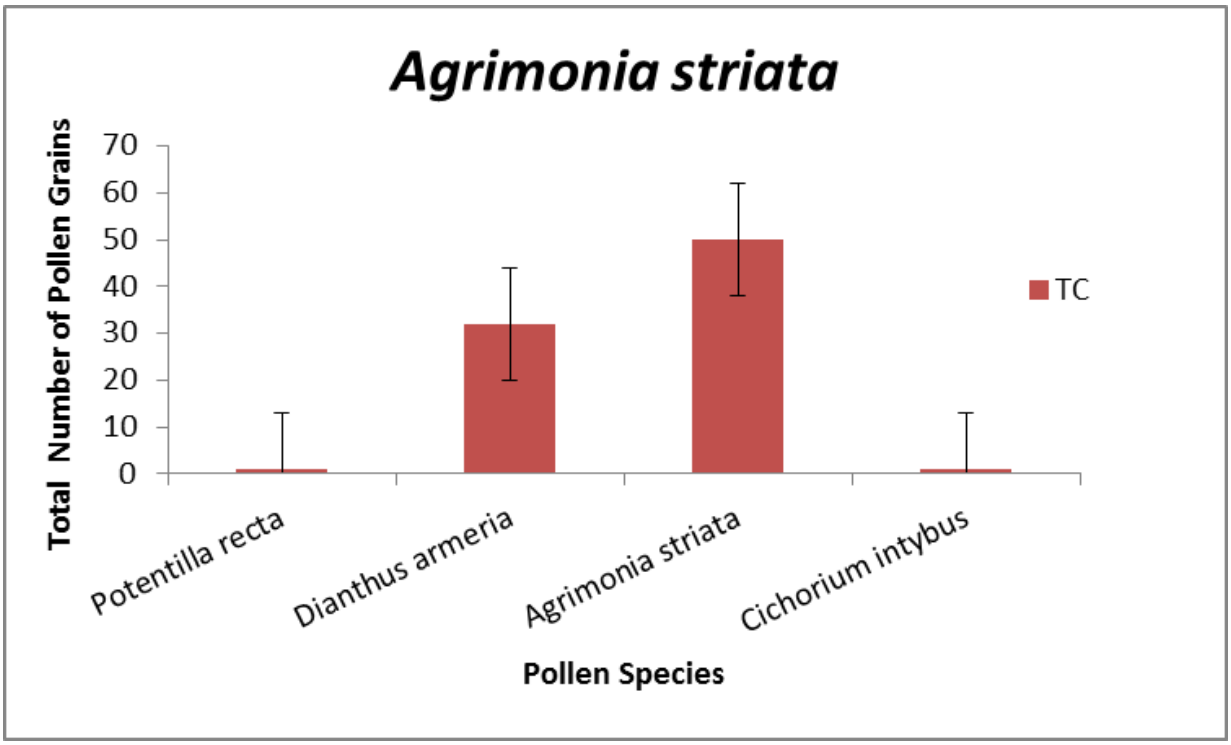

Figure 10 Total number of pollen grains identified on *Agrimonia striata* Stigmas at Terra Cotta (n=3). Error bars represent standard error

Potentilla recta

Potentilla recta displayed the third largest pollen diversity value for the study (Table 5). The diversity value was not considered significantly different due to its occurrence at only 1 site. *Ranunculus acris* deposited the largest quantity of pollen (heterospecific). Conspecific pollen was the second largest pollen deposition found on *Potentilla recta* (Figure 11). The remaining two heterospecific pollen grains, *Hypericum perforatum* and *Tragopogon dubius* represented a small quantity of deposited pollen. *Potentilla recta* is the only species where the largest pollen deposited was comprised of heterospecific origin. The pollen depositional pattern found on this stigmas species demonstrates an undesirable pattern for native stigmas species. Since both the stigmas species and pollen species are exotics, this specific heterospecific pollen deposition is not concerning, although future studies should be suspicious if this pattern arises with native stigma species.

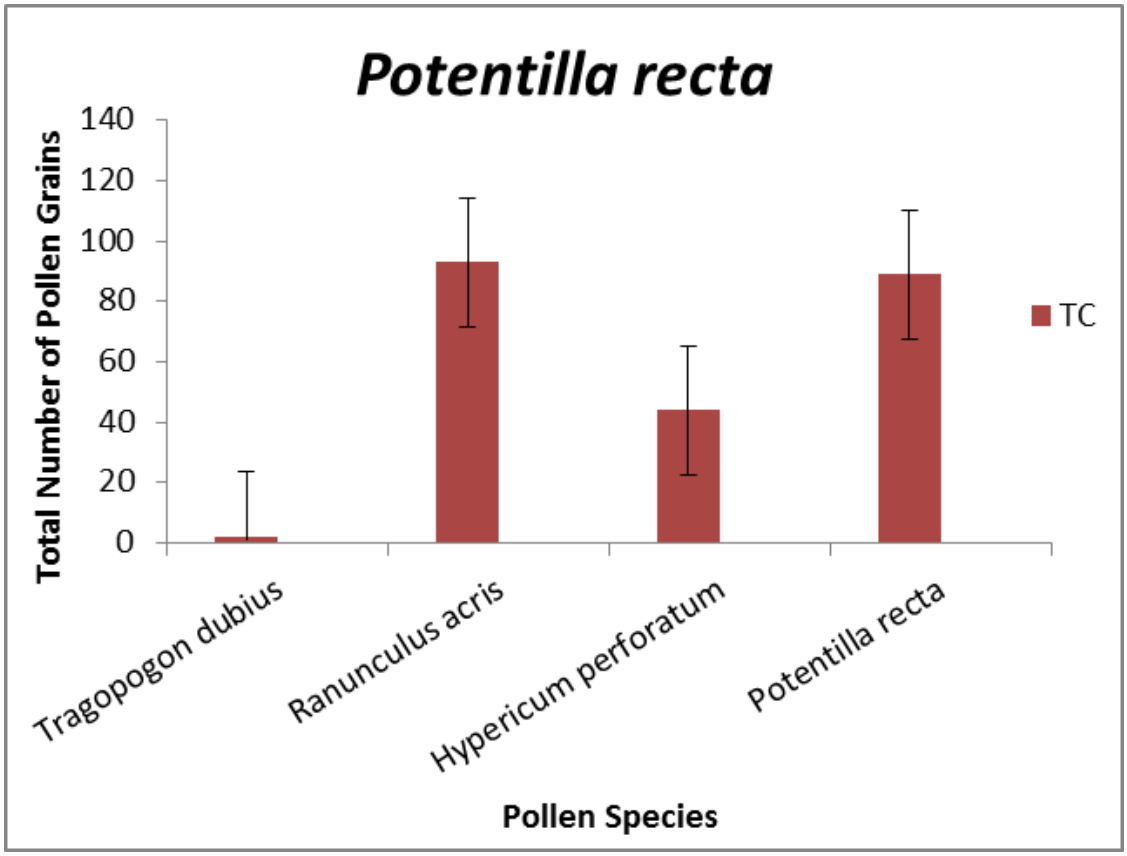

Figure 11 Total number of pollen grains identified on *Potentilla recta* stigmas at Terra Cotta (n=4). Error bars represent standard error

4.5 Pollinator Observations

The majority of insects observed visiting flowers were bee species at all sites over the entire sampling period (Figure 12). Flies comprised the second most common visitor but were observed less than half as often as bees (Figure 12). Wasps and Butterflies were observed in similar quantities. The "other" category is comprised of ants, beetles, and insects that could not be easily identified. Terra Cotta had the highest number of pollinator observations, whereas Upper Credit had the fewest pollinators observed.

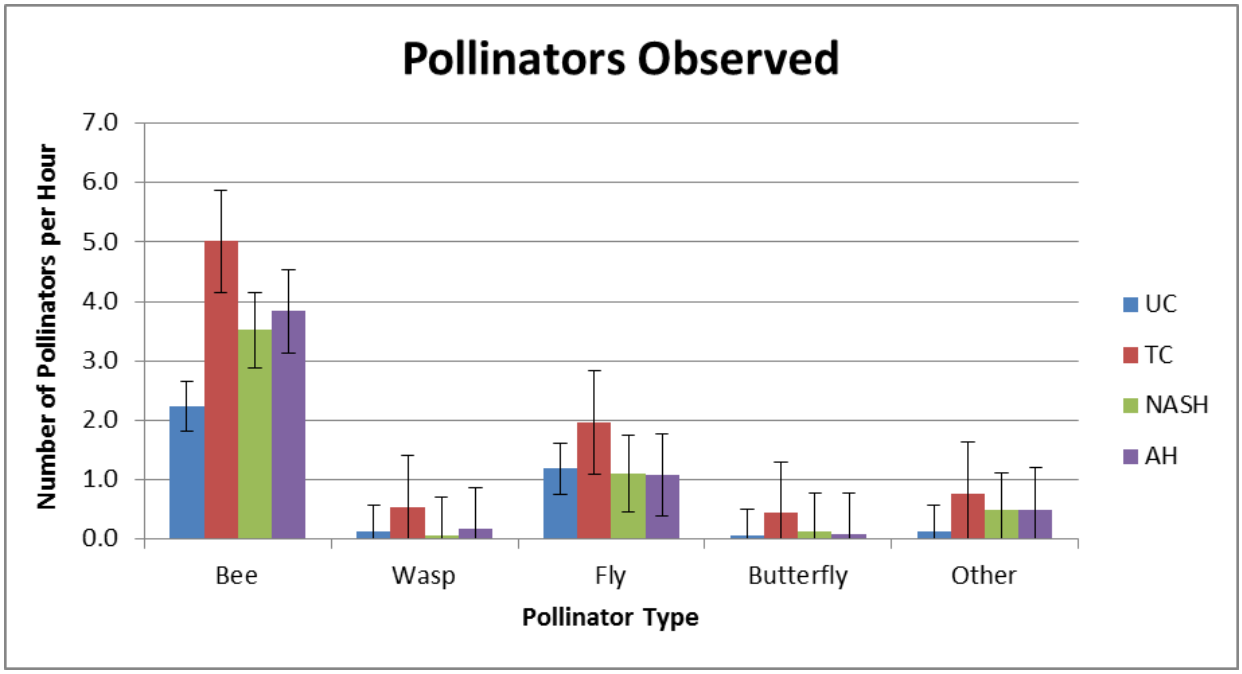

Figure 12 Pollinator Observations at all Sites during the Sampling Season. n=279. Error bars represent standard error

5.0 Discussion

This study quantified multi-species pollen deposition for meadow ecosystems in southern Ontario, establishing baseline community data using observations from four replicate locations. These methods create a viable, expedient, and accurate protocol for multi-year plant-pollinator studies realistic for staff-limited Conservation Authorities. Notwithstanding *Potentilla recta* and *Cirsium* species stigma results, exotic pollen in 2011 did not represent the majority of pollen deposited onto native plant stigmas; even though within all meadows, native plant species represented on average 32 % of the plant community. These depositional patterns seem to indicate that native plant reproduction may not be negatively affected by the large presence of exotic species and associated pollen found within this study. This pattern is encouraging and future monitoring can determine if changes to pollen deposition occurs and take appropriate restoration actions. Continuing pollen deposition monitoring would give Conservation Authorities important data to help fulfill their mandate of conserving biodiversity without requiring several staff members to execute the protocol. Future annual compilation of pollen deposition data can also aid in helping to create a comprehensive conservation and restoration plan based on the plant species present within Conservation Authorities' meadows, if in future it becomes the goal of either Conservation Authority to increase native plant species within meadows.

5.1 Pollen dispersal throughout Ontario meadow communities

The majority of pollen deposited on most stigmas was conspecific. Although heterospecific pollen was observed on stigmas in varying quantities (Table 5) the diversity of pollen grains deposited on stigmas is low according to the Shannon Index results present by

Magurran (2004). Magurran (2004) reported that Shannon Diversity values typically range between 1.5-3.5, where 3.5 is usually the upper limit of the diversity value in plant communities; however, there are no current instances where the Shannon Diversity Index has been used on pollen depositional studies. Utilizing Magurran (2004) range of values, species within this study with relatively high diversity values are not considered high on the Shannon Diversity scale. Although many pollen deposition studies present data in a variety of formats (McLernon et al. 1996, Larson et al. 2006, Goodell et al. 2010, Dietzsch et al. 2011a, McKinney and Goodell 2011), utilizing the Shannon index within the pollen deposition literature would allow for direct comparisons between studies, rather than the current state of the literature where few studies present data in a standard format. Given that this is the first study that analyses the pollen depositional diversity with the Shannon Diversity Index, Magurran (2004) values may not apply. Utilizing the Shannon Diversity Index can aid in understanding pollen deposition equitability and from that knowledge specific interesting patterns can be investigated further. Since the Shannon Diversity Index is widely used by conservationists (Mouillot and Lepretre 1999, Mendes et al. 2008), its values can likely be comprehended by a multitude of conservation staff members. All stigmas displayed diversity values below 2 with the highest diversity value (1.76) found on *Cirsium* species stigmas (Table 5 and Figure 6). The low diversity and resulting low heterospecific pollen deposition observed in this study suggests pollinators displayed a high floral constancy at each study site. Lower heterospecific pollen deposition usually results in higher pollination success as defined by increased seed set (Morales and Traveset 2008). Jakobsson et al. (2008) determined 1-4 pollen grains per stigma did not negatively affect seed set while Flanagan et al. (2009) found 25 heterospecific pollen grains per stigma did not negatively affect seed set. Both studies classified these values as very low to low heterospecific pollen

transfer (see further Moragues and Traveset (2005), Levin and Kerster (1967), Campbell and Motten (1985), Bosch and Waser (1999), Bartomeus et al. (2008)). Conversely, Thomson et al. (1982) found quantities of 5-10 heterospecific pollen grains negatively affected seed set; however, the heterospecific pollen grains were allelopathic in nature. *Agrimonia striata* displayed the highest diversity value (0.78) and only received on average 11 heterospecific pollen grains per stigma (Figure 10). Given the low quantities of heterospecific pollen found on native stigmas within this study it is unlikely these values would negatively affect seed set. The low heterospecific pollen deposition found in this study may reflect the lack of congeneric exotic or invasive species present within any of the meadows. Brown et al. (2002) and Kandori et al. (2009) both observed frequent interspecific pollinator movements between a native and invasive congener (*Lythrum alatum* and invasive *L. salicaria; Taraxacum japonicum* and the invasive *T. officinale*, respectively) and therefore a higher probability of heterospecific pollen transfer between an invasive and native plant species and/or reduced visitation to native plant species. With low heterospecific pollen deposition on most plant species in this study, reproduction rates for either exotic or native plant species may not be affected significantly.

Native stigmas within this study received little heterospecific pollen, with the largest diversity found on *Agrimonia striata* (0.78). Three other native plant species (*Aster, Eupatorium perforatum, Rudbeckia hirta*) had very low diversity indices (< 0.17). Low diversity indices may potentially favour native plant reproduction due to reduced pollen competition from large quantities of heterospecific pollen (given that all other factors are equal). Hybridization between *Aster/ Symphyotrichum* and *Solidago/Oligoneuron* species are common (Goodwin 1937, Semple and Brammall 1982, Semple et al. 1992, Chmielewski and Semple 2003) making pollen species level identification challenging. The ability for hybridization in these species suggests that some

of the conspecific pollen noted in this study could be heterospecific. Hybridization was not quantified in this study. Given the high abundance of exotic plant species within each meadow, the observed low diversity scores indicated favourable conditions for native species populations because of low heterospecific pollen deposition competition and seemingly high floral constancy. If conspecific pollen continues to represent the majority of deposited pollen on native plant species then the threat to their long term viability may be lower than initially perceived (in regards to heterospecific pollen deposition) within similar meadows in Ontario where there is a strong presence of the observed exotic species.

Exotic species within this study (*Achillea millefolium*, C*entaurea jacea*, *Dianthus armeria*, *Dipsacus fullonum*, *Lotus corniculatus*) were deposited solely by conspecific pollen similar to native species. Of the eight exotic species that received heterospecific pollen (*Agrimonia striata*, *Cirsium* species, *Daucus carota*, *Hypericum perforatum*, *Melilotus alba*, *Potentilla recta*, *Ranunculus acris*, and *Vicia cracca*) many species had limited heterospecific pollen, which could favour exotic plant reproduction due to low heterospecific pollen competition. Exotic species (*Cirsium*, *Hypericum perforatum*, and *Potentilla recta*) displayed the four highest and both *Cirsium* and *Hypericum perforatum* had significantly different pollen diversity depositions compared to native species. Larger pollen diversity depositions (Figure 6) could be advantageous for native plant reproduction because heterospecific pollen deposition could potentially decrease the seed set of these exotic species, thereby potentially reducing their rates of reproduction; however, reproduction rates were not studied and further investigation is needed to fully determine what specific quantity of heterospecific pollen impacts seed set for these species.

5.2 Potential threats to southern Ontario meadows from invasive plant species found within this study

While the low abundance of heterospecific pollen is advantageous, the degree to which exotic species found within this study are invasive may also affect the pollination success of natives. The exotic species found in this study are further examined to aid in determining species removal priorities during restorations.

Cirsium arvense is considered a moderate invasive exotic (Canadian Wildlife Service 1999) or a Category 1, aggressive invasive exotic, (Urban Forest Associates Inc. 2002). Conspecific pollen loss on *Cirsium* stigmas is minimal thus the large deposition of exotic heterospecific pollen is potentially beneficial to native species. Conspecific pollen loss can decrease the number of conspecific pollen grains arriving at these exotic species' stigma and therefore potentially reduce reproduction rates of exotic plant species due to pollen limitation (Morales and Traveset 2008, Flanagan et al. 2009). Since native plant species pollen was not found as heterospecific pollen their conspecific pollen loss is less. With small CPL there may be more conspecific pollen arriving at native species' stigmas and therefore their reproduction rate is less hindered from this specific mechanism.

The presence of exotic pollen deposited on *Cirsium* stigmas may be indicative of *Cirsium* species' facilitative nature in a future invasive species meltdown (Simberloff and Von Holle 1999). If pollinators visit between *Cirsium* species and other exotic species more frequently than native plants, native plants may have reduced conspecific pollen load deposition due to reduced visitation rates, while exotic species visited by pollinators are benefited from increased visitation. *Vicia cracca* pollen represented the largest quantity of pollen deposited on *Cirsium* species stigmas at Upper Credit and small quantities of pollen at both Terra Cotta and Nashville.

Although *Cirsium* and *Vicia cracca* have contrasting floral shapes (Actinomorphic disk flowers (Moore 1975) and Zygomorphic (Aarssen et al. 1986), respectively), pollen flow between both species is observed within these meadows. Both species have purple flowers and pollinators, such as *Apis. mellifera,* often forage based on colour preference (Hill et al. 1997). A. *mellifera* are known pollinators of *Cirsium arvense* (Moore 1975) and *Vicia* species can be pollinated by wild bees, bumblebees, and *A. mellifera* (Aarssen et al. 1986). Pollen flow between *Cirsium* species and *Vicia cracca* is likely due to visitation from *A. mellifera* in these meadows due to floral colour similarities and not floral shapes. This is the first documented occurrence of pollen flow between these species. Similar pollen deposition from *Cirsium* species is not observed on *Vicia cracca* stigmas (Figure 7 and Appendix A) and therefore several variables/mechanisms require further investigation to explain pollen deposition differences such as flower timing, density, plant visitation, or pollen placement on pollinator bodies due to differences in floral shapes.

Additionally, rhizomes are the main reproduction mechanism of *C. arvense* and seed dispersal is secondary (Alberta Agriculture and Rural Development 2013); consequently, frequent visits by pollinators are unnecessary for its reproduction and therefore mainly reduce visitation to native plants. *Cirsium arvense* has been found to facilitate pollinator visits to surrounding native plants only at floral densities less than 2.5 inflorescences (floral heads) m^{-2} (Ghazoul 2006) and once that density surpassed there was a negative effect on floral visitors to focal native plants. Due to the main reproduction mechanism of *C. arvense* and its' invasive competitive nature for resources, *C. arvense* can likely produce higher inflorescence rates than 2.5 m^{-2} when unmanaged, thereby decreasing its facilitative effect and increasing its competitive effect. This study did not measure the inflorescences per m^{-2} and therefore cannot accurately state

whether the findings in Ghazoul (2006) are likely to occur within these meadows but Conservation Authorities should consider the potential negative effect *C. arvense* may have in future. Due to its aggressive invasive nature and probable competition for pollinator visits, *Cirsium* species, specifically, *C. arvense*, may pose a threat to native meadow biodiversity in future even though its pollen was not found in high quantities on native species' stigmas in this study. Increased densities of invasive and exotic species may negatively affect native plant pollination biology. Monitoring pollen movement within ecosystems can aid in understanding when/if a threshold is crossed where invasive or exotic species inhibit or decrease native plant reproduction and therefore informed adaptive management decisions can be made promptly.

Melilotus alba is considered a moderate invasive (Canadian Wildlife Service 1999); however, *Vicia cracca, Melilotus alba,* and *Lotus corniculatus* are considered highly invasive exotic species (Category 2) within southern Ontario (Urban Forest Associates Inc. 2002). Both Urban Forest Associates Inc. (2002) and Canadian Wildlife Service (1999) utilized literature reviews, as well as practitioners surveys and experiences to rank invasive species and only differ based on resources consulted. Both *Vicia cracca* and *Melilotus alba* displayed some heterospecific pollen deposition within this study (Table 5 and Figure 9). *Vicia cracca* has a fatty substances used for self-fertilization (Aarssen et al. 1986) and heterospecific pollen grains may easily become attached from *Bombus* species' bodies during visitation. It is unknown if the fatty substance or pollinator visitation patterns are responsible for the small exotic heterospecific pollen deposition observed on *Vicia cracca* stigmas (See Appendix A). Turkington et al. (1978) determined that *Melilotus alba* attracts a variety of pollinators (bees, wasps, and flies) and therefore visitation rates by multiple types of pollinators (even if they display high individual floral constancy) may result in a large diversity of pollen grain deposition for *Melilotus alba*.

Unlike *Vicia cracca*, which was mainly deposited with other exotic species' pollen, high pollen deposition from native plants was observed on *Melilotus alba* stigmas (Figure 9). Murphy and Aarssen (1989) previously observed negative effects on pollen germination of four plants likely due to pollen pH differences in *Melilotus alba* (8.8) and *Vicia cracca* (7.1) heterospecific pollen. Large pH differences in pollen may present an allelopathic-like effect (Murphy and Aarssen 1989) and thus both exotic species' pollen may reduce germination rates in native plant species. *Aster spp*. and *Oenothera biennis* were deposited with very small quantities of *Melilotus alba* and *Vicia cracca* respectively. However, small quantities may be sufficient to affect reproduction rates due to these potential interactions.

Further investigation is required to determine if these exotic plant species negatively affect native plant reproduction due to their allelopathic-like tendencies. Allelopathic studies would require isolating specific chemicals (Murphy et al. 2009a); however allelopathic studies are difficult due to the multitude of chemicals within pollen and time consuming while attempting to isolate specific chemicals to test them (Murphy et al. 2009a). The pollination syndrome of *Vicia cracca*'s influence on native plant pollination may be reduced at these study sites. The pollen deposition observed on *Melilotus alba* stigmas and its invasive competitive nature may reduce native plant populations, specifically *Agrimonia striata* (Figure 9), and should be further investigated or removed from sites, if possible. *Lotus corniculatus* is only effectively pollinated by *Bombus* species, due to its floral shape (Jones and Turkington 1986), which may explain the low pollen deposition diversity. Although *L. corniculatus* is considered a highly invasive exotic species (Category 2), due to its pollination requirements (bilaterally symmetrical floral shape requiring larger *Bombus* species to effectively pollinate it (Jones and Turkington 1986) *L. corniculatus* possesses a low ability to deposit pollen on native plant species and

conversely, native pollen has a low potential of being deposited onto its stigma. Therefore, the absence of *L. corniculatus* on Environment Canada's invasive species list is more probable in this scenario than the "highly invasive exotic" category. The immediate removal of *L. corniculatus* is likely unnecessary.

Dipsacus fullonum is considered an exotic species that is moderately invasive (Category 3) (Urban Forest Associates Inc. 2002); however it is not considered invasive by Canadian Wildlife Service (Canadian Wildlife Service 1999). It did not display any heterospecific pollen deposition within this study and therefore does not seem to pose a threat to native pollination success through pollen loss or deposition. *D. fullonum* pollen grains were not found on any stigmas, native or exotic, besides itself and therefore *D. fullonum* does not seem to pose a threat to native plant pollination through pollen transfer mechanisms.

Hypericum perforatum is considered a moderate invasive exotic plant (Canadian Wildlife Service 1999) or a Category 4 with little to no threat of invasiveness (Urban Forest Associates Inc. 2002). *Linaria vulgaris* is also considered an exotic species with little to no threat of invasiveness, Category 4 (Urban Forest Associates Inc. 2002) and absent from any Environment Canada listing (Canadian Wildlife Service 1999). *Linaria vulgaris* was almost entirely deposited with conspecific pollen (Table 3) and it is mainly pollinated by *Bombus* species due to the constricted nature of its' floral shape (Saner et al. 1995). This constricted flower shape and limited pollen transfer indicates that *Linaria vulgaris* may not pose a threat to native pollination from Heterospecific Pollen Deposition. *Hypericum perforatum* has two high diversity values at two different sites (Table 3) and its pollen grains are observed on several other stigmas suggesting that visiting pollinators may not be displaying floral constancy. Most of the heterospecific pollen found on *H. perforatum* stigmas were from other exotic species and

therefore native conspecific pollen loss was negligible. *Hypericum perforatum* pollen was only found on stigmas of two native plant species (*Rudbeckia hirta* and *Clinopodium vulgare*) in low quantities (See Appendix A). *Hypericum perforatum* also flowers from late June through until mid-August (Crompton et al. 1988) and therefore the large depositional diversity may be due to the length of the flowering season rather than a significantly larger quantity of pollinator visits. Since the majority of plants in the study sites were exotic, it is difficult to determine if pollinators would visit a similar number of native species and mirror the heterospecific pollen found on the stigmas of *H. perforatum* in meadows dominated by native species. Future heterospecific pollen studies within these meadows should monitor this species to adequately determine its invasive potential.

5.3 Comparison to previous studies of heterospecific pollen transfer

There are a range of outcomes of heterospecific pollen transfer (i.e. low amounts of heterospecific pollen transfer) (Bosch and Waser 1999, Moragues and Traveset 2005, Bartomeus et al. 2008) versus large quantities of heterospecific pollen transfer (Brown and Kodric-Brown 1979, McLernon et al. 1996). The low heterospecific pollen deposition found within this study is hypothesized to occur due to the lack of invasive congenerics, low floral diversity within the studied meadows resulting in few plants with sequential flowering times (i.e. flowering one after another not concurrently), and potential differences in plant-pollinator communities amongst previous studies or a combination of these above factors.

Studies that have reported high heterospecific pollen deposition usually quantify the effect of a congeneric invasive on a native plant species (Brown and Mitchell 2001, Memmott and Waser 2002, Kandori et al. 2009). For example the invasive *Lythrum salicaria* has a negative effect on the native *L. alatum* by reducing visitation rates to the native *L. alatum* when present

(Brown and Mitchell 2001). A similar result was observed by Kandori et al. (2009) between the native *Taraxacum japonicum* and the invasive species *T. officinale,* where reduced visitation and seed set was observed in *T. japonicum* in the presence of the invasive *T. officinale*. Studies reporting low heterospecific pollen transfer contain invasive and native plants that are not congenerics. For example both Moragues and Traveset (2005) and Bartomeus et al. (2008) found low deposition from the highly invasive *Carpobrotus* species on several different native plant species, where none were congenerics.

Larson et al. (2006) found similar results where their focal native plant species were mainly deposited with conspecific pollen grains even within infested invasive *Euphorbia esula* sites. The likelihood of pollinator movement between invasive and native species seems increased when both plants are congeneric and therefore the potential for heterospecific pollen transfer may also be increased. Within this study there were no invasive and native congenerics observed.

The proportion of heterospecific pollen on most stigma species in this study is lower than reported from a 1-year study done in Kingston, Ontario that documented heterospecific pollen deposition in a mid-successional abandoned farm field (McLernon et al. 1996). Although there are only four similar stigma species between McLernon et al. (1996) and this study, three of the four species in this study had lower heterospecific pollen deposition than what was reported by McLernon et al. (1996). McLernon et al. (1996) reported 21 common entomophilic species within their study, whereas this study had 14 or fewer species per site and therefore differences in the density and diversity of species within meadow communities may affect the heterospecific pollen deposition quantity even between sites in a similar geographic region. High heterospecific pollen transfer was observed in multiple species by Fang and Shuang-Quan (2013) in a

community of over 100 flowering native species with no invasive species; a more diverse meadow with increased floral choices may increase the likelihood of heterospecific pollen deposition because pollinators' floral constancy could be difficult to maintain in an array of flowers with similar colours or shapes. Bartomeus et al. (2008) and Moragues and Traveset (2005) community studies reported low heterospecific pollen on focal plant species that comprise of 10 species or less, which is a similar plant composition quantity to this study. Low plant species diversity within meadows also reduces the likelihood of sequentially flowering species, thereby reducing the opportunity for interspecific pollinator movement. *Cirsium arvense*, *Hypericum perforatum*, and *Melilotus alba* flower throughout the sampling period in this study (Moore 1975, Turkington et al. 1978, Crompton et al. 1988) thereby increasing the likelihood of HPD (in conjunction with other features such as pollinator visitation and floral morphology).

Community composition of plants and pollinators vary spatially and temporally and usually require several sampling seasons to obtain an accurate representation (Roubik 2001, Williams et al. 2001). Therefore, differences in either plant composition or pollinator composition may affect the pollen deposition patterns and account for differences in heterospecific pollen deposition between studies. Differences in heterospecific pollen deposition may also vary due to specific pollinator species and their foraging choices at each site. Since both McLernon et al. (1996) and this study utilized one season of field data, precise conclusions about their different results is difficult. High heterospecific pollen transfer is often observed in anemophily (wind) pollinated species (Murphy and Aarssen 1989) potentially due to the large quantities of pollen available for outcrossing, as well as the reliance on weather (wind) rather than pollinators for successful outcrossing (Murphy and Aarssen 1989, Murphy and Aarssen 1995a). Due to the entomophilic nature of the plants within this study (Table 1 and Table 2), the

potential for heterospecific pollen deposition may be decreased. Difference between study site characteristics could make the generalizing of plant-pollinator communities difficult not only on a broad scale but at a smaller scale for southern Ontario meadows.

5.4 Native floral diversity and density

One of the value-added outcomes of my study was a clear documentation of the low native floral diversity within all sampled Ontario meadows (Table 2 and Table 3). Delany et al. (2000) listed approximately 40 common native flora species that can be found in meadows within this study region and the species found within this study represents approximately 25 % (10 of 40) of the potential native meadow species. It appears that the historical lack of meadow management in southern Ontario may have affected meadow composition, resulting in the dominance by exotic species. If some active management actions are not undertaken, the few remaining meadows within Ontario may eventually comprise entirely of exotic species, providing no real conservation purpose. Diverse floral resources in meadows facilitate pollinator visitation both when floral resources are congeneric (Thomson 1978, Moeller 2004) and when they differ in floral morphologies (Ghazoul 2006); however, the presence of exotic or invasive species does not necessarily promote similar facilitative visitation patterns. Although meadows within this study may be considered diverse, the exotic species representing the diversity do not guarantee positive visitation rates to native species (Ghazoul 2006). Future efforts should be made to enhance native meadow plant diversity within Ontario meadows.

Pollinator visitation rates to meadows have been shown to be related to floral density (Kunin 1997, Bosch and Waser 1999), where higher densities attract a higher number of floral visitors. Williams et al. (2011) found that bees' use of plants in four habitat types correlated with plant floral density in both native and exotic plants. Less dense flower patches produced a lower

seed set, even with similar pollen deposition quantity, than highly dense flower patches (Bosch and Waser 1999) and therefore floral density could alter plant reproduction rates. Floral density could also affect conspecific pollen loss, as fewer pollen grains are deposited on conspecific stigmas thereby compounding the effect of heterospecific pollen deposition. Moeller (2004) observed that conspecific pollen deposition was positively related to conspecific flower density. Differences in floral densities may also explain the heterospecific pollen deposition differences in scientific studies. Floral density might affect the seed set in sparse areas, such as the meadows found in this study, because of reduced genetic diversity and therefore a reduction in the number of viable seeds produced, due to geitonogamy. Floral density was not measured in this study and therefore concrete conclusions cannot be drawn about floral density within Ontario meadows; however, future pollen studies should consider including floral density in their experimental design if time, personnel, and funds are available.

5.5 Assessing current heterospecific pollen transfer

Studies assessing the proportion or quantity of heterospecific pollen that impedes native pollen fertilization are not extensive (Bosch and Waser 1999, Bjerknes et al. 2007). Several studies have shown seed output from heterospecific pollen deposition results in a decreased seed set relative to pure conspecific pollen composition (Brown and Mitchell 2001, Moragues and Traveset 2005, Wilkinson 2008). Galen and Gregory (1989) illustrated that hand pollination studies can deposit 1-2 orders of magnitude more pollen than found under natural entomophilous conditions (Galen and Newport 1988). Although some studies have controlled the extent of heterospecific hand pollination to reflect natural conditions (Murphy et al. 2009a, Murphy et al. 2009b) direct conclusions made from hand pollination studies that do not subsequently report on natural pollination conditions could overstate the negative effects of heterospecific pollen

deposition under natural entomophilous pollination conditions. Several studies found far fewer quantities or proportions of heterospecific pollen deposition during natural pollination (Bosch and Waser 1999, Moragues and Traveset 2005, Bartomeus et al. 2008); therefore, it is difficult to determine if these natural conditions would also result in a reduced seed set and if so, under what circumstances. Murphy et al. (2009a) found as few as four heterospecific pollen grains reduced pollen tube generation due to allelopathic effects; however allelopathic effects of all exotic and native species are not known and allelopathic effects are a less common IPT mechanism (Murphy et al. 2009b). With such discrepancies in the literature, it is difficult to determine what quantity of heterospecific pollen will negatively affect native plant species reproduction and therefore assess whether the observed heterospecific pollen deposition in this study is detrimental or conversely, has little or no effect on seed production. Concurrent studies assessing pollen deposition patterns in conjunction with seed set within natural pollination would aid in this predicament.

As researchers continue to understand the effects of heterospecific pollen on pollination success, efforts should be made to understand what proportion of a stigma surface covered in heterospecific pollen decreases reproduction and pollination success for different plant species. By quantifying pollination success for different plant species along with better assessments, either through in-field seed set success studies or attempting hand pollination controlling efforts, of potential heterospecific pollen deposition by invasive species, a more comprehensive understanding of the effect invasive species have on native plant communities will be gained. Without more knowledge about this multifaceted topic it will be impossible to generally predict the impact of small or moderate deposits of exotic heterospecific pollen (as found in this study) on seed set within diverse native plant communities.

5.6 Pollinator constancy may affect depositional patterns

Low heterospecific pollen deposition is usually associated with pollinator specialization (Waser 1986) or at least floral constancy (Waser 1998, Chittka et al. 1999). This study found the most abundant pollinators at all sites were bees. Most bees are floral generalists able to utilize floral resources from a variety of plant species (Michener 2000); however, several studies highlight the ability of bee species to display floral constancy within a foraging session (Goulson 1994, Stimec et al. 1997, Raine and Chittka 2005, Flanagan et al. 2009). A higher floral constancy could promote higher depositions of conspecific pollen and lower depositions of heterospecific pollen, which was observed in this study. Furthermore, Grixti and Packer (2006) determined that 79 % of pollinators at a site near Forks of the Credit were generalists, suggesting that the majority of pollinators at Upper Credit and Terra Cotta, both under 20 km away, could have similar pollinator trends. Pollinator constancy could potentially further explain the differences between heterospecific pollen deposition in this study and the study by McLernon et al. (1996). Pollinator constancy can vary with genera and species, for example *A. mellifera* can associate certain floral colours and scents with rewards and some *Bombus* species can learn efficient probing mechanisms for complex flowers (Kevan and Baker 1983). Kevan and Baker (1983) reviewed floral constancy within insects and summarized that *A. mellifera* displayed floral constancy once they learned to recognize a flower with sufficient rewards, while some *Bombus* species were shown to have conditional constancy where flowers are categorized, and their foraging rate depends on factors such as floral density.

Grixti and Packer (2006) determine the 7 most abundant bee species at their Forks of the Credit study site: *Ceratina (Zadontomerus) calcarata* Robertson, *Lasioglossum (Lasioglossum) zonulum* (Smith) (exotic species), *L. (Dialictus) lineatulum* (Crawford), *L. (Evylaeus) cinctipes*

(Provancher), *Halictus (Halictus) ligatus* Say, *Andrena (Taeniandrena) wilkella* (Kirby)(exotic species), and *Colletes inaequalis* Say. These species may also represent a large proportion of bees found within Upper Credit and Terra Cotta; however, due to the spatial and temporal variation in pollinator populations findings from Grixti and Packer (2006) may not represent exact species composition within this study. Furthermore, this study did not perform species level identification unlike Grixti and Packer (2006), thus making direct comparisons impossible.

Further studies documenting pollinator species in Ontario meadows are needed to make more accurate generalizations about specific bee species behaviour; however based on the small diversity of heterospecific pollen, the pollinators present within these meadows seem to exhibit high floral constancy, potentially suggesting a large proportion of *A. mellifera* or other bee species that largely display floral constancy.

5.7 Effects of exotic plants on native pollinators

Invasive or exotic plant species often have higher floral densities and greater nectar reward than native plant species (Brown and Mitchell 2001, Chittka and Schurkens 2001) and therefore are presumed to attract more pollinators to the general area. This attraction may or may not negatively affect native plant pollination success depending on several factors as discussed above; however, pollen is used as a nutritional source for many pollinators and the effect of exotic pollen nutrition on native bees is worth understanding. Although under natural conditions only a few exotic species may pose a threat to the pollination success of native plant species due to IPT, the effect of exotic pollen on the health of pollinators is unknown (Stout and Morales 2009). Pollen analyses have shown ranges of nutritional content such as protein, amino acids, and lipids (Roulston and Cane 2000, Pernal and Currie 2001). There is limited research done on pollen nutritional composition (Roulston et al. 2000b, Pernal and Currie 2001) due to chemical

analytical difficulties (Roulston and Cane 2000). Exotic pollen might have a negative effect on the health of native pollinators, for example, *Melilotus alba* and *Cirsium arvense* have low protein content (Pernal and Currie 2001), which can produce lower pollinator body size (Schmidt et al. 1987, Roulston and Cane 2002) and reduced colony size (Di Pasquale et al. 2013). These negative effects may reduce native pollinator populations, consequently reducing visitation rates to native plants and increasing the likelihood for pollen limitation; however, little research has been conducted on the pollen quality required for native bees (Muller et al. 2006, Stout and Morales 2009) and therefore potential effects on native plant reproduction are speculative. Currently, little is known about the effect of invasive plant species' nectar or pollen on the pollinator community in southern Ontario (Richards et al. 2011); however, determining pollen transfer patterns between species increases the knowledge of pollinator behaviour within southern Ontario.

Although most of the species sampled in this study were exotic (Table 2), some exotic plants are well established within Ontario. Some exotic species naturalization in an area and are incorporated into the ecosystem without inflicting increased competitive effects on native species (Richardson et al. 2000b, Colautti and MacIsaac 2004, Richardson and Pyšek 2012). Climate, reproductive traits, residence time, dispersal traits, and supply of propagules are factors that affect exotic species naturalization (Richardson and Pyšek 2012). It is a combination of these factors as well as specific species that influences naturalization (Richardson and Pyšek 2012); thus it is difficult to assign a specific threshold. Exotics have been known to be utilized by pollinators, for example *Daucus carota* is a known source of pollen for pollinator species *Hylaeus dialictus* and *Lasioglossum dialictus* (Richards et al. 2011) and *Dipsacus fullonum* has been shown to be a food source and nesting site for some *Bombus* and *Ceratina* species

(Vickruck et al. 2010). Although the plants within each study site are comprised mainly of exotic species, some may potentially aid pollinator populations and therefore, knowledge about pollinators utilizing established exotic plants should be further investigated prior to large scale removal of all exotic plant species (see further Figure 13 and 14).

5.8 Current state of Ontario meadows and restoration recommendations

All meadows sampled in this study were not actively restored and have been allowed to undergo succession after anthropogenic influences. The floral composition and richness for meadows found in this study is a concern for the biodiversity of meadows in southern Ontario. Given that approximately 10 % of both the TRCA's (Toronto and Region Conservation Authority 2007) and the CVC's (Credit Valley Conservation 2009) managed land is considered meadow, the native meadow biodiversity in both Conservation Authorities is low given this study's findings. Delany et al. (2000) listed 39 meadow genera with 16 specifically mentioned as core species. This study found 7 native plant genera comprising 17 % of the total potential meadow genera with 25 % of the core genera represented. As most of the land designated for conservation in Ontario falls within the system of Conservation Authorities, it seems likely that the patterns observed within Conservation Authority-managed conservation areas may apply broadly to other meadow ecosystems throughout the province. The low diversity observed in meadows within both Conservation Authorities may be exacerbated when considering their meadow definitions include mowed grass and agricultural fields. CVC defines meadows as "land cover that is in a state of natural regeneration after cultural or human-based disturbances, such as an abandoned farm field." (Credit Valley Conservation 2009) and therefore the two study sites within CVC can be considered a typical meadow within CVC jurisdiction. If these meadows are typical, then 10 % of all meadows within CVC boundaries will most likely have a high

abundance of exotic species with few meadows actually conserving native meadow biodiversity, an ecosystem already threatened across Ontario. TRCA defines meadows as "including sand barren, savannah and tallgrass prairie[s]" while also allowing for " "cultural" or "anthropogenic" natural communities, for example, old fields, but not manicured lawns"(Toronto and Region Conservation Authority 2007). Their definition is more specific than CVC's and encompasses the threatened savannah and tallgrass prairie ecosystem. TRCA may therefore have higher quality meadows within their jurisdiction compared to CVC but TRCA also allows for areas to be deemed meadows if they are "cultural old fields," which resembles the description for the meadows sampled in this study. It is difficult to determine at what percentage cultural old fields make up TRCA's 10 % designated meadow land.

Forest, wetland, and water quality targets are cited in several planning and management documents (City of Toronto 2000, Toronto and Region Conservation Authority 2007, City of Toronto 2010). Conservation Authorities utilize Official Plans and Municipal documents for natural heritage planning within their watersheds (Toronto and Region Conservation Authority 2007). With specific targets emphasized (written within documents) subsequent reports require updates on achieving stated targets. In contrast to "Significant Woodlands", "Significant Wetlands", and "Significant Valleylands" meadows and prairies are usually not a natural heritage feature explicitly described (Government of Ontario 2014). Additionally, in urbanized areas trees provide air purification and shade that most residents desire in natural areas (Conservation 2011) whereas, meadows do not seem as desirable for some residents, as they do not provide an escape from heat during hikes in the summer (Conservation 2011). Government reports and public desires highlight forestry targets, which guide some funding actions for Conservation Authorities; however, since meadows are a threatened ecosystem in Ontario,

Conservation Authorities should conserve biodiversity within meadows as part of their mandate of biodiversity conservation. Reporting specifically on meadow ecosystems may influence future government documents and public opinion polls.

5.9 Recommendations for creating biologically diverse meadows in Ontario

In order to better accomplish their mandate, Conservation Authorities, especially within the Carolinian zone and transitional zone of Great Lakes-St. Lawrence, should convert existing old fields, abandoned land, and open cut grass areas into patches of meadows. Previous restoration documents cite that native species will eventually overcome exotic species in meadows and exotic species removal is unnecessary (Woodland et al. 1995); however, the high presence of exotic species found within this study suggests this approach may not be effective for all meadows in southern Ontario. Evidently there will be instances when converting horticultural grassed areas is counter intuitive to its current use, for example campsites or underneath picnic tables but there are several underutilized open areas that can be seeded to create small patches of meadows (personal observation). Furthermore, Parks Canada (2008) does not recommend natural regeneration of old agricultural fields as a measure to create biodiverse meadows as it can increase exotic species establishment. Seeding small patches to create meadows is also supported by this study as little native biodiversity was observed within these meadows. In addition, natural regeneration assessments should be undertaken for newly acquired land and where there is a long history of agricultural use, active seeding and restoration actions should be planned immediately in properties, funds permitting, thereby reducing the likelihood of exotic or invasive species establishment. Seeding or planting areas adjacent to picnic areas, such as Terra Cotta, could provide an excellent vista for picnic users, as well as providing small meadows within the Conservation Authority landscape. Converting current mowed areas to

either open restored meadows (preferred) or managed pollinator gardens with boundaries between plant species (the latter providing a manicured up- kept appearance) can provide better function and biodiversity within Conservation Authorities than mowed grass.

Increasing native plant exposure to Conservation Authority visitors may also indirectly promote the use of native plants within their own personal gardens, thereby further increasing native plant abundance regionally. Signs and informative pamphlets can be used as an additional opportunity to educate the public on the use of native species and the detrimental effects of invasive species. Small patches of naturalized areas can increase pollinator abundance within urbanized settings (Tommasi et al. 2004, Matteson et al. 2008) and by converting existing mowed grass areas, the native diversity and abundance of plants and pollinators may be increased without having to sacrifice as many large areas designated for forest restoration. Naturalized areas/gardens also utilize less water than manicured lawns and therefore fewer resources (water and gas) for Conservation Authorities, furthering their mandate and brand. Unfortunately, due to the Endangered Species Act (2007), Conservation Authorities cannot propagate threatened or endangered plant species, but perhaps the creation of a more functional meadow habitat may allow for natural colonization of threatened or endangered plant species in the future (Parks Canada 2008).

Conservation Authorities, especially within the Carolinian and Great Lakes-St. Lawrence zones, should increase their efforts to broaden their restoration and management goals to include actively managed meadows that will promote native plant establishment and continuance. Within their management goals, disturbance regimes should be considered (Parks Canada 2008). Conservation Authorities should plan for controlled disturbances within their meadows to reduce invasive species and prevent encroachment from tree species unless their long term goal for that

area is forest succession. By creating and managing for functional meadows, the 10 % of area currently designated as meadows within each Conservation Authority will provide a higher degree of meadow biodiversity, thereby increasing their ability to accomplish their mandate.

5.9.1 Utilizing plant-pollinator interactions to restore Ontario meadows

For successful meadow restoration, it is not sufficient to simply plant native species, but to also be aware of the species present and their life stages' needs. Several pollinators require certain plants for different life stages (Kremen et al. 2007), for example butterflies require nectar plants for food resource and host plants for larva development (Cane and Tepedino 2001). Some exotic plants can be toxic to larva development and become a sink for populations as they become utilized by butterflies (Graves and Shapiro 2003) and therefore careful consideration of plant species should be undertaken.

The utility of exotic species by native pollinators has been documented in some studies (Graves and Shapiro 2003, Tepedino et al. 2008) and therefore quick removal of exotic species may result in a decline in existing pollinator populations (Gibson et al. 2006, Carvalheiro et al. 2008). In order to improve meadow plant-pollinator biodiversity, planting and seeding of native species should be done within existing fields, without the immediate removal of all exotic or invasive species (Parks Canada 2008). Immediate removal will decrease floral abundance and nectar reward for pollinators, potentially reducing visitation rates or decreasing current pollinator populations due to lack of nutrition quantity. For example, Conservation Authorities should determine the equivalent native plant that would provide adequate pollen for *Hylaeus Dialictus* and *Lasioglossum Dialictus* if the decision by a Conservation Authority is to remove *Daucus carota* from meadow communities. Exotic plant removal ideally should not be undertaken until

an equal population of native species exists, to avoid pollinator population crashes, making the total net loss to the meadow, zero.

Currently, TRCA utilizes community restoration days to restore some of their current meadows (Figure 20) (Figure 21), which consists of weeding and disposing of *Cirsium* species (Toronto and Region Conservation Authority 2012). This action of weeding, while noble, may have a negative impact on the meadow due to loss of floral density and potential food or nesting sources for pollinators (Graves and Shapiro 2003, Gibson et al. 2006). Community restoration days are a common occurrence within Conservation Authorities as a way to engage the community in their natural areas, and they are recommended by Parks Canada for ecological restoration (Parks Canada 2008). Slight changes to community restoration days to improve meadow restoration could be made to include planting using plugs while weeding and seeding small patches at a time over the course of two or three years. By working at smaller spatial scales over the course of a few years, native plants can become well established and invasive and exotic plant populations can been diminished thus providing consistent floral resources throughout the season (Gibson et al. 2006, Aldridge et al. 2011, McKinney and Goodell 2011) adjacent to new nesting sites.

Figure 13 A meadow within Toronto Region Conservation Authority prior to a Community Restoration Day. Adapted from http://www.trca.on.ca/the-living-city/watersheds/etobicoke-mimico-creek/creektime/archive/?id=142575

Figure 14 A meadow within Toronto Region Conservation Authority after a Community Restoration Day. Adapted from http://www.trca.on.ca/the-living-city/watersheds/etobicoke-mimico-creek/creektime/archive/?id=142575

In meadows that have the majority of floral composition from native plants, immediate removal of exotic species might be the preferred decision if the exotic plant species function within the meadow is redundant and therefore would not harm pollinator populations (Parks Canada 2008). Without an in-depth visitation network analysis of each meadow in Ontario it is difficult to generalize which species to always immediately remove for all Ontario meadows based on this study, therefore ensuring native seedling and planting occurs while current exotic plants are still established would aid in maintaining current pollinator populations.

Sequentially flowering native plants should also be considered for meadow restoration plans. By providing floral resources throughout the growing seasons, pollinator populations can be better maintained (Gibson et al. 2006, Morales and Aizen 2006), thereby aiding in long term restoration of meadows. Invasive plants are hypothesized to facilitate pollination when flowering times are not during native plant flowering times (Waser and Real 1979); however, if there are not adequate native plant species that can flower sequentially, exotic species with certain attributes might be the best alternative to increase floral resources for pollinator populations. The desirable attributes of these exotic species include: mainly deposited by conspecific pollen, with little to no heterospecific pollen deposition on native plants, and possessing no threat of invasiveness through other competitive effects; however, using exotic plants should only occur as a last resort and after numerous studies assessing their pollen and potential Interspecific Pollen Transfer potential.

Plants should also be chosen based on current pollinator populations (Parks Canada 2008, Menz et al. 2011). Choosing plants with preferred floral shapes and colours could aid in maintaining pollinator populations and allow for exotic plants to be phased out and eventually eliminated. Bees are known to visit a range of flower shapes but mainly prefer bilateral

symmetry (Proctor et al. 1996) and are known to prefer yellow or purple/blue flowers (Proctor et al. 1996). This study found a large population of bees within all four sites, as well as, that the majority of flowers, native and exotic, were yellow or purple/blue, therefore native plants with yellow or purple/blue flowers should be emphasized in initial restoration planting plants. Insects' ability to discriminate between floral colours is greater than humans (Kevan and Baker 1983) and their ability to discriminate between patterns and outlines is approximately an order of magnitude less than ours (Kevan and Baker 1983). Consequently, Conservation Authorities should take into consideration the differences in vision between insects and humans when choosing plants.

Conservation Authorities have the opportunity to aid in conserving wild plant and pollinator populations with changes to their current approach to maintaining meadows, as well as initiating active meadow restoration within their jurisdiction. With annual plant-pollinator interaction monitoring they can constantly assess their current meadows and adapt their restoration and conservation methods accordingly.

6.0 Conclusion

The majority of meadows within this study were comprised of exotic plants with few interspersed native plant species. Although the majority of plants were exotic, pollen deposition on both exotic and native plants were chiefly conspecific pollen, suggesting constancy in foraging patterns by pollinators and potentially low negative effects from heterospecific pollen transfer. Some species observed (*Cirsium* species, *Vicia cracca*, *Melilotus alba,* and *Hypericum perforatum*) can be considered invasive or pose a threat to native plant pollination success and therefore their removal or management should be a higher priority.

Typically, urbanized areas are comprised of many invasive and exotic species and the meadows within Conservation Authorities in southern Ontario followed this trend. The exotic species within these studied southern Ontario meadows were well-established and comprised 68 % of observed plant species richness. Full restoration of the present meadows to a historical state is time consuming, requires large labour costs, and may realistically be unfeasible given the disturbance level (Hobbs et al. 2009). Due to such restrictions Conservation Authorities should adapt their restoration practices to focus on creating hybrid or novel meadow ecosystems (Seastedt et al. 2008, Hobbs et al. 2009). These ecosystems would support both a collection of native and exotic plants, as well as the pollinators that rely on the meadow ecosystem for floral resources and nesting sites. Restoration plans for these ecosystems should focus on maintaining a variety of floral resources for pollinators throughout the Spring and Summer seasons, while gradually eliminating exotic species that seem to pose a threat to pollination systems given this study's findings (i.e. *Cirsium arvense, Melilotus alba*, and potentially *Vicia cracca* and *Hypericum perforatum*). When choosing plants to remove and plant during restoration activities, consideration should be placed on a plants' function within the system as required by pollinators

throughout their lifecycles. Flowering times and flower types should be considered when selecting plants for restoration plans based on current meadow plant and pollinator populations and relationships. In newly acquired properties destined for meadow habitat, especially old agricultural fields, Conservation Authorities should draft restoration or rehabilitation plans instead of allowing natural regeneration to occur in old agricultural fields because the latter approach aids in the establishment of exotic species. Land donated or acquired that has a well-established meadow with thriving native plant species would have different long term management plans than properties acquired that have been heavily disturbed. Disturbed properties would require more active restoration activities spanning several years than remnant undisturbed meadows where the control of invasive or exotic species is more manageable and feasible.

Due to the temporal and spatial variability of plant-pollinator communities, historical or restored meadows within Conservation Authorities should be monitored annually for pollen dispositional patterns and species assemblages. Monitoring will allow Conservation Authorities to alter their actions according to their results and to therefore better manage their plant and pollinator species, thus better achieving their objective of biodiversity conservation. If time, personnel and funds are adequate, parameters such as floral density, seed set and pollinator species should be considered additional useful parameters to understand and characterize plant-pollinator communities within meadows in southern Ontario. Although the integration of meadow areas into the long term plan of Conservation Authorities is worthy, the lack of management or restoration actions has created meadows that are largely dominated by exotic species and therefore conserve little native plant biodiversity. Furthermore, the exotic species present may not necessarily be providing adequate resources to the pollinator communities that

rely on them. Conservation Authorities need to implement small restorative efforts sequentially to reduce the exotic plants and to reintroduce a subset of native plants back into their meadow ecosystems.

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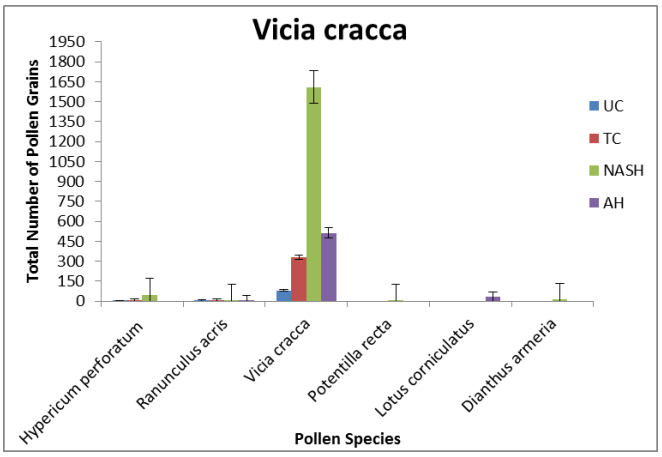
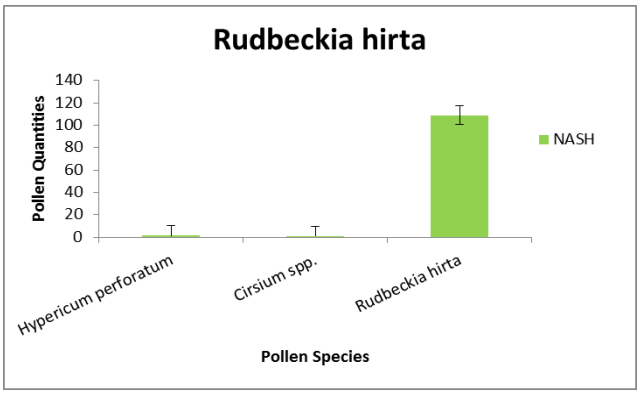
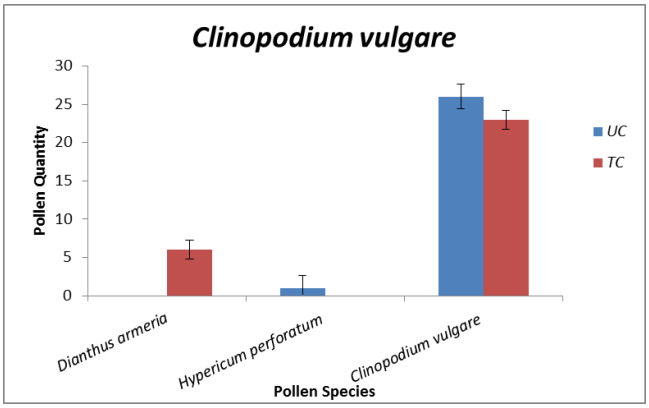
Appendix A Total number of pollen grains deposited on *Vicia cracca*, *Rudbeckia hirta*, and *Clinopodium vulgare* stigmas



Pollen Quantities Found on *Vicia cracca* Stigmas at all Sites



Pollen Quantities Found on *Rudbeckia hirta* Stigmas at Nashville



Pollen Quantities Found on *Clinopodium vulgare* Stigmas at Upper Credit and Terra Cotta